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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXVII. The Mechanism of Regurgitation of Duodenal Contents into the Stomach

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Boldyreff's[1] statement that antiperistalsis in the duodenum is the mechanism of the regurgitation of intestinal contents into the stomach was the first instance in which antiperistalsis had been reported as a normal motor activity of any part of the gastro-intestinal tract, except, of course, the ascending and transverse colon. Accordingly, under the direction of Dr. Carlson, we attempted to verify this statement of Boldyreff's and to determine, if possible, additional facts bearing on this problem. The literature on this particular point appears to be confined to two papers by Boldyreff. In the first article[2] he says that when oil and acid are introduced into the stomach, duodenal regurgitation occurs by a "mechanism of its own." To support his later designation[1] of duodenal antiperistalsis as the mechanism of this regurgitation, he published no experimental data.

METHODS OF EXPERIMENTATION

I. Aseptic section of vagi and splanchnics in dogs, with comparison of regurgitation before operation and after recovery.

II. Surviving excised cats' stomachs with duodenum and jejunum in oxygenated Tyrodi's solution. Observation of movements when 0.5 per cent HCl was introduced into the stomach through oesophagus.

[1] Boldyreff: Quart. Journ. Exper. Physiol., 1914, viii, 8.
[2] Boldyreff: Ruskii Vrach, 1904, iii, 1305.

III. Fluoroscopic examination of cat's duodenum filled via jejunal fistula with $BiONO_3$ suspension, when 0.5 HCl was introduced into the stomach.

IV. Direct observation of movements of gastro-intestinal tract exposed under Tyrodi's solution at 37°C. and the effect upon these movements of the introduction of 0.5 HCl into the stomach through the oesophagus before and after the section of the vagi and splanchnics.

RESULTS

Method I of course required a series of experiments on a given dog before operation to determine the normal frequency of duodenal regurgitation when 0.5 per cent HCl had been introduced into the stomach. The procedure was as follows:

1. 150 cc. 0.9 per cent NaCl solution into stomach by oesophageal tube. Aspirated after 30 minutes—color noted.

2. 150 cc. 0.5 per cent HCl into stomach by tube. Aspirated after thirty minutes—color noted.

If the first removed sample was colorless, and the second showed any appreciable yellow tinge, this was considered a positive result; i.e., regurgitation had occurred. Whereas, if the recovered acid was colorless, it was considered a negative result.

It was decided that the color of the sample was a sufficiently delicate test for the presence of bile after it had been found that one drop of bile added to 100 cc. of water stained it a bright straw color.

It soon became apparent, however, that regurgitation under these conditions was the exception rather than the rule. Consequently nerve section was an unsatisfactory method of attacking the problem, because, with an irregular normal, it would be difficult to interpret the post-operative results. Accordingly this method was largely abandoned. We continued, however, to accumulate a large number of tests on normal dogs to determine in what percentage of cases regurgitation occurred under our stated conditions. The results were:

Using 15 dogs and testing them on an average of about five times each, a total of 76 trials:

150 cc. 0.9 per cent NaCl in stomach for thirty minutes caused no regurgitation in 100 per cent of 22 cases. Therefore, in later trials, the stomach was only washed with salt solution to determine possible presence of bile before the acid was introduced. 150 cc. of 0.5 per cent HCl in stomach for fifteen minutes = regurgitation positive in 6 out of 20 trials = 30 per cent positive. 150 cc. 0.5 per cent HCl in stomach

thirty minutes = regurgitation positive in 15 out of 36 trials = 43 per cent positive. The results of 20 additional trials were discarded because of emesis, or on account of the presence of bile in the stomach contents before the introduction of acid.

When regurgitation occurred, judging by the pale color of the returned acid, but very little duodenal contents could have entered the stomach. In spite of this fact, the acidity was usually reduced from 0.5 per cent to about 0.35 per cent HCl. This would seem to indicate that *duodenal regurgitation is not the factor of greatest importance in the reduction of the high acidity of the stomach contents;* for it is difficult to conceive of much pancreatic juice passing back through the duodenum without becoming mixed with bile.

Nine experiments on 4 dogs with sectioned splanchnics yielded 100 per cent positive regurgitation. In these cases the acid removed after thirty minutes contained much bile; and the acidity averaged only 0.237.

A man with a gastric fistula (Mr. V) being available, two series of tests were run to determine in what percentage of cases acid in the stomach caused regurgitation in man. These results are shown in the accompanying Tables I and II. In Table I it is seen that the appetite secretion of the gastric juice accumulated during twenty minutes' chewing food, and amounting to an average of 32.6 cc. with an average acidity of 0.411 per cent, caused regurgitation in 40 per cent of 10 cases.

Method II after five trials was also discarded because of the spasm of the pylorus induced, as described by Cannon,[3] by section of the jejunum. This, of course, prevented the escape of gastric contents into the duodenum, and also made regurgitation impossible.

Method III (fluoroscopy) proved useless because the introduction of $BiONO_3$ suspension through a jejunal fistula caused pyloric spasm. Moreover, the bismuth, acting as a foreign substance itself caused duodenal contractions.

Method IV was followed on 11 cats and 1 dog with the following results.

1. 100 cc. 0.9 per cent NaCl introduced into the stomach via oesophagus induced no movements in the other duodenum and no regurgitation into the stomach.

2. In the 1 dog and in 9 out of the 11 cats 100 cc. 0.5 per cent HCl introduced into the stomach by tube via oesophagus induced rhyth-

[3] Cannon, W. B.: Mechanical factors of digestion, p. 126.

TABLE I

Regurgitation of duodenal contents into stomach in man (Mr. V.) from the presence in the stomach of appetite gastric juice

DATE	200 cc. H_2O IN STOMACH ONE HOUR BEFORE CHEWING FOOD		APPETITE—SECRETION DURING 20 MINUTES CHEWING		REMARKS
	Amount color	HCl acidity	Amount color	HCl acidity	
		per cent		*per cent*	
1. July 19	18 cc. Very pale yellow	0.14 HCl	55 cc. Pale lemon color	0.407 HCl	Positive
2. 20	21 cc. Cloudy Opalescent	0.131	43 cc. Trace bile Clear opalescent	0.362	Faintly positive
3. 21	22 cc. Cloudy No bile	0.177	35 cc. Clearer Trace bile	0.462	Faintly positive
4. 22	17 cc. Very cloudy No bile	0.124	38 cc. Cloudy No bile	0.242	Negative
5. 23	24 cc. Colorless	0.096	28 cc. Colorless	0.411	Negative
6. 26	18 cc. Very cloudy No bile	0.255	40 cc. Fairly clear No bile	0.447	Negative
7. 27	26 cc. Cloudy (food?)	0.141	25 cc. Clear No bile	0.409	Negative
8. 29	18 cc. Opalescent	0.073	17.5 cc. Clear No bile	0.489	Negative
9. 30	20 cc. Clear	0.064	25 cc. Faint yellow	0.420	Positive
10. 31	21 cc.	0.130	30 cc. Clear—colorless	0.461	Negative
Average	20.3 cc.	0.134	32.6 cc.	0.411	40% Positive

TABLE II

100 cc. 0.4 per cent HCl *into stomach of Mr. V. causes no regurgitation (after 20 minutes)*

DATE	200 cc. H_2O INTO STOMACH AT 10.00 A. M. EMPTIED AT 11.00 A. M.		100 cc. 0.4 % HCl INTO STOMACH AT 11.00 A. M. EMPTIED AT 11.20 A. M.		REMARKS
	Amount and color	HCl acidity	Amount and color	HCl acidity	
		per cent		*per cent*	
1. Aug. 9	12 cc. Clear Colorless	0.298	50 cc. Clear Colorless	0.395	Negative
2. 10	20 cc. Clear Colorless	0.306	24 cc. Clear Colorless	0.380	Negative
3. 12	11 cc. Clear Colorless	0.213	33 cc. Clear Colorless	0.362	Negative
4. 13	20 cc. Clear Colorless	0.266	39 cc. Clear Colorless	0.365	Negative
5. 17	21 cc. Cloudy Yellow	0.343	36 cc. Clear Colorless	0.351	Negative
6. 18	18 cc. Clear Colorless	0.252	41 cc. Clear Colorless	0.379	Negative
7. 19	25 cc. Clear	0.175	40 cc. Clear	0.369	Negative
8. 20	14 cc. Clear	0.237	34 cc. Clear	0.377	Negative
9. 23	16 cc. Clear	0.220	15 cc. Clear	0.319	Negative
10. 24	18 cc. Clear	0.241	26 cc. Clear	0.387	Negative
Average	17.5 cc.	0.2551	33.8 cc.	0.3629	100% Negative

mical pulsations and segmentation movements of the upper duodenum. In all of these animals regurgitation of duodenal contents into the stomach had taken place, as shown by the withdrawal of the stomach contents after thirty minutes.

3. In 2 of the cats the upper duodenum remained quiescent for at least thirty minutes after the introduction of 100 cc. 0.5 per cent HCl into the stomach, and the stomach contents of these two animals showed no intestinal regurgitation.

4. Actual antiperistalsis of the upper duodenum was never observed.

5. In the case of the 10 animals showing movements of the upper duodenum and intestinal regurgitation the average amount of the 100 cc. acid recovered after thirty minutes was 95.7 cc. with an acidity of 0.382 per cent. The 2 cats showing neither duodenal movements nor regurgitation yielded an average of 92 cc. with 0.44 per cent acidity.

6. In 5 cats with section of both vagi nerves in the neck 100 cc. 0.5 per cent HCl in the stomach caused similar movements of the upper duodenum and intestinal regurgitation in 4 cases, and no movements in the duodenum and no regurgitation in 1 case. In all these experiments the acid remained in the stomach thirty minutes.

7. In 4 cats with section of all the splanchnic nerves 100 cc. 0.5 per cent HCl in the stomach induced the above movements in the upper duodenum and regurgitation of intestinal contents in each case.

A brief description of the pulsating movements referred to in the above summary as invariably accompanying regurgitation and constantly absent when no regurgitation occurred, may be of interest. These movements occurred in the portion of the duodenum between the pyloric sphincter and a point just below the entrance of the common duct—a region corresponding with what Cole[4] has named the "pilleus ventriculi." Here two types of movements were noted:

1. Multiple, periodic, deep, constricting rings occurring throughout this region, but mainly in the lower half of the first portion of the duodenum. These constrictions did not pass along as peristaltic waves; but rather resembled marked, slow, segmentation movements.

2. Peristaltic waves, usually deep, originating about 4 per minute in a pulsating ring just distal to the pyloric sphincter, and passing rather rapidly down to disappear in a tonic constriction just beyond the opening of the common duct.

Occasionally a third type of movement was noted in this region. The entire duodenum above the common bile duct was tightly con-

[4] Cole: Jour. Amer. Med. Assoc., 1913, lxi, 762.

tracted as a whole, the walls appearing anemic—so strong was the constriction. This spasm lasted usually about one minute.

These movements began in from three to twenty-five minutes (average thirteen minutes) after the introduction of 0.5 per cent HCl into the stomach. They did not appear continuously throughout any experiment, but a period of gradually diminishing motor activity would be followed by a short period of rest.

Granting that the pyloric sphincter is relaxed occasionally (the presence of the strong acid on the gastric side should assure this) it is easy to see how any one of these three types of movements in the upper duodenum would be a mechanism capable of forcing back some duodenal contents into the stomach.

In general the amount of regurgitation appeared directly proportional to the degree and duration of the movements in the first portion of the duodenum. Antiperistalsis was never seen in the duodenum. This is in accord with Starling's[5] and Cannon's statement that "an antiperistalsis is never observed in the small intestine." No movements of the upper duodenum were ever noted except as described above as invariably accompanying regurgitation following the introduction of acid into the stomach (or when 0.5 per cent HCl was introduced directly into the duodenum, as described in a succeeding paragraph.) This statement agrees with that of Holzknecht[6] that peristalsis is rarely seen in the upper duodenum.

It may, of course, be objected that these observations were made upon animals under ether anaesthesia, and are therefore not truly normal. But a very light anaesthesia was always maintained. Moreover, the frequent observation of normal peristalsis in the stomach and small intestines, antiperistalsis in the ascending colon, and segmentation movements in the small intestine would seem to show that the motor activities of the gut were little impaired.

Having determined that the peculiar movements described above occurred following the introduction of 0.5 per cent HCl into the stomach even in the absence of extrinsic nervous connections, the following experiments were performed in an attempt to further localize the mechanism of regurgitation:

Cat under Tyrode's solution (as in Method IV).

[5] Starling, E. H.: Recent advances in the physiology of digestion, Chicago, 1907, p. 142. Cannon, Loc. cit., p. 142.

[6] Holzknecht and Jones: "Der Radiologische Diagnostik," etc., Wien. 1908, p. 17.

1. 100 cc. 0.5 per cent HCl into stomach per oesophagus.

2. Stomach emptied of acid contents and washed with 0.9 per cent NaCl soon after characteristic movements had started in the upper duodenum. (Regurgitation noted as faintly positive.)

3. After upper duodenum had become quiet and remained so ten minutes, 5 cc. 0.9 per cent NaCl was injected into the lumen of the upper duodenum with a very fine hypodermic needle. No movements of upper duodenum noted during following fifteen minutes.

4. 5 cc. 0.5 per cent HCl injected by needle into the lumen of upper duodenum. Characteristic pulsating movements noted after four minutes and continuing about ten minutes. Repetitions of this experiment on 3 other cats gave the same results.

This experiment shows that the discharge of the strong acid from the stomach into the upper duodenum initiates the movements in that region through local mechanisms.

CONCLUSIONS

I. 150 cc. 0.5 per cent HCl left in dogs' stomachs for fifteen and thirty minutes caused regurgitation in 38 per cent of 56 trials. In man, an average of 32.6 cc. gastric juice (acidity 0.411 per cent) accumulated during twenty minutes' chewing of food, caused regurgitation in 40 per cent of 10 trials. Whereas, 100 cc. of 0.4 per cent HCl retained in stomach for twenty minutes caused no regurgitation in 100 per cent of 10 other cases.

II. Duodenal regurgitation following the introduction of 0.5 per cent HCl into the cat's stomach is effected by means of characteristic movements (mostly "rhythmical pulsations" or "segmentation movements"[7]) in the first portion of the duodenum, initiated by the passage of strong HCl into the duodenum, and occurring in the absence of extrinsic nervous connections. Regurgitation never occurred in the absence of these movements.

III. Antiperistalsis of the duodenum is not concerned in this regurgitation.

[7] Cannon: Loc. cit., p. 142.

THE ORIGIN OF THE PROTEOLYTIC FERMENTS OF THE BLOOD

The Question of the Specific Character of Certain Ferments

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Since the publication by Abderhalden (1) of his experiments on the "Protective ferments of the animal organism" and especially on the sero-diagnosis of pregnancy a very large literature has developed. The major part of this literature has concerned itself with the pregnancy reaction. The results obtained have been conflicting. Some workers have verified completely all data reported by Abderhalden while others have been unable to confirm the experiments.

The basis for the reaction is briefly this (2). Chorionic cells and substances not in harmony with the maternal blood get into the maternal blood stream. The presence of such cells and substances causes the development of an enzyme or ferment which is able to split placental protein.

Abderhalden and his students have steadfastly maintained the absolute specificity of the reaction. They contend that the blood of pregnant individuals contains a ferment which is specific for placental protein and that such an enzyme or enzymes with like power do not exist in normal blood serum or in the blood serum from other than pregnant source. Furthermore they believe the reaction to be a digestion of the substrate and not an autodigestion of the proteins of the serum by enzymes freed from an anti-enzyme inhibition. The recent work of Michaelis and Lagermarck (3), Flatow (4), Herzfield (5) and of others abroad, and of Williams and Pearce (6), Falls (7), Jobling, Eggstein and Petersen (8) and Bronfenbrenner (9) in this country brings the position of Abderhalden into serious doubt.

From the work of these authors and others the existence of polyvalent enzymes in the blood serum is certain. These polyvalent enzymes are proteolytic in character. They may either be increased in amount

or in power of action under certain conditions. It is clear, therefore, that the value of the Abderhalden reaction will be apparent only when there is a quantitative differentiation between results obtained by the action of these enzymes and the so-called specific ferment of pregnancy.

The first worker to suggest this essential point was Kjaergaard (10) in May, 1914. In June, 1914 at the symposium on the subject before the American Medical Association (11) the same suggestion was made independently by Dr. Carlson.

It was with a view to reducing the Abderhalden reaction to a quantitative test and thereby to make its differential value greater that this investigation was carried out.

Early results showed that a differentiation on the strength of color produced with ninhydrin was unreliable. Ninhydrin is so easily affected by the physical and mechanical factors of the method as to make questionable the slight differences in depth of color. A greater or less color cannot always be assigned to a greater or less degree of digestion. The other quantitative method involved the time factor.

EXPERIMENTAL METHODS

In addition to the methods first suggested by Abderhalden, *i.e.*, the dialysis and optic methods (12), the following have since been suggested (a) Williams and Pearce coagulation method (loc. cit.), (b) the Van Slyke method (13), and (c) the very recent method of Kiutsi discussed and modified by Malone (14). Of these methods we have employed two: the simple method of Williams and Pearce and the dialysis method of Abderhalden.

The Williams and Pearce method was altered in but one respect. This involved the readjustment of the volume of the filtrate obtained after coagulation with acetic acid up to 15 cc. In the dialysis method thimbles No. 679a were boiled for fifteen seconds, inverted onto filter paper, allowed to cool and then handled with sterile forceps. One-half gram of the tissue desired was added and on to this from 1–1½ cc. of serum was allowed to flow from a sterile pipette. The contents was covered with a layer of toluol and placed in the special dialyzing flasks furnished by the Arthur H. Thomas Co. The outer liquid was covered with a thin layer of toluol and the whole incubated at 37–38° for a definite interval of time (16–20–44 hours). The controls varied. In every case an equal amount of serum was used as one control. At regular intervals all substrates were tested. Frequently substrate plus sterile

salt solution was used as control. In all the early tests with the Williams and Pearce method a control of placenta or other substrate and inactivated serum was used. In many instances all of these controls were run.

As to the preparation of the substrate some change was made from Abderhalden's directions purely in the way of safeguarding the reaction. Abderhalden advises boiling the substrate until a test with 5 cc. of the clear filtrate fails to give a reaction with 1 cc. of a 1 per cent solution of ninhydrin. We boil the material three times after the test is no longer given for about five minutes each time but do not, however, continue to boil beyond seven times. On removal from the containing jar the tissue is washed with distilled water, pressed between filter paper and then weighed. The requisite quantity of tissue is put into a beaker and boiled twice with about 50–75 cc. of water. Between boilings the tissue is rinsed with distilled water and after the second boiling is placed upon filter paper. Sterile sheets cover all working surfaces. Other details are as given by Abderhalden.

EXPERIMENTS

These consisted of the following series.

A. Action of blood serum from pregnant individuals on human placenta.

B. Action of blood serum from pregnant individuals on various tissues.

C. Action of blood serum from normal individuals on human placenta.

D. Action of blood serum from various pathological conditions on human placenta.

A. THE ACTION OF BLOOD SERUM FROM PREGNANT INDIVIDUALS ON HUMAN PLACENTA

The results of the tests on serum from 14 pregnant individuals using the Williams and Pearce technic are summarized in Table I. Tests were made with serum from 23 other pregnants using the dialysis method with the results given in Table II. An additional series of tests were made on a pregnant goat with the results shown in Table III.

TABLE I

Action of blood serum from pregnant women and pregnant dogs upon human placenta using the Williams and Pearce technic*

SOURCE OF SERUM	NO. OF TESTS	POSITIVE	NEGATIVE	TIME
				hours
Women in ninth month.....	10	10	0	20
Dogs 1–5 days before term..	4	4	0	20

*The preparation of a satisfactory substrate from the placenta of dogs is very difficult. Following a suggestion of Abderhalden (15) human placenta has been used throughout.

TABLE II

Action of blood serum from pregnant women and pregnant dogs upon human placenta using the dialysis method

SOURCE OF SERUM	NO. OF TESTS	POSITIVE	NEGATIVE	TIME
				hours
Women in ninth month.....	5 excl. of preliminary tests	5	0	20
Healthy pregnant dogs.....	18	16	2	20–24

In one case a series was run on dog on arrival at laboratory. Serum very fatty. Absolutely negative results.

TABLE III

Action of the blood serum of pregnant goat upon human placenta, extending over $7\frac{1}{2}$ weeks. Dialysis method

DATE	AMOUNT OF SERUM	NO. OF TESTS	POSITIVE	NEGATIVE	TIME
	cc.				*hours*
February 22....	1	4	4	0	20
March 11.....	1	1	0	1	20
March 15.....	1 1	1 1	0 1	1 0	20 40
April 14.....	1 1.5–2	1 2	0 2	1 0	20 20

*Two kids were born on April 17

B. THE ACTION OF BLOOD SERUM FROM PREGNANT INDIVIDUALS ON VARIOUS TISSUES

The tissues used were prepared by transfusing the dogs with normal salt solution until the organs were practically blood free and then grinding and boiling as given in Abderhalden's directions. The results are summarized in Table IV.

TABLE IV

Action of blood serum from pregnant women, pregnant dogs and a pregnant goat on dog pancreas, liver and kidney

TISSUE	NUMBER OF TESTS	AMOUNT OF SERUM	POSITIVE	NEGATIVE	TIME
		cc.			
Pancreas	3 human 7 dog	1.5	3 6	1	20–22
Liver	1 goat 5 dog	1.5	5	1 dog	20–22
Kidney	4 dog	1.5	1	3	20–22
Summary*	20		15	5	

*Excludes dog which gave negative with placenta. All reactions with this same serum on pancreas, liver and kidney were negative.

In the series some of the tests were run parallel with those upon placenta. Opportunity was thus afforded to compare the strength of reaction. In the majority of instances placenta gave the deeper color. While this might indicate a specificity of a definite sort, still, as mentioned before, too much importance cannot be put upon mere depth of color.

It seemed especially important at this point to determine (a) whether there exist in the blood serum of *normal* individuals enzymes which can produce dialyzable products with placental protein and (b) if such enzymes do exist can we differentiate between the polyvalent enzymes and the specific enzymes of pregnancy by any quantitative method.

C. THE ACTION OF BLOOD SERUM FROM NORMAL INDIVIDUALS ON HUMAN PLACENTA

It was later found that the method we employed had been used previously by Kjaregaard (loc. cit). It involved the extension of the

time of incubation from twenty to forty-four hours. The limit of forty-four hours was placed after carrying out a series of tests for twenty, twenty-four, thirty, thirty-six, forty, forty-four-hour periods. The results are given in Table V. Similar tests were carried out using dog kidney, liver and pancreas as substrate with practically identical results.

TABLE V

Action of blood serum of normal individuals on human placenta for 20 to 44 hour periods

SOURCE OF SERUM	NUMBER OF TESTS	TIME	POSITIVE	NEGATIVE
		hours		
Male dogs.....	14	20–22	0	14
Male dogs.....	16	40–44	14	2
Human male....	4	20	0	4
Human male....	4	40–44	4	0

D. THE ACTION OF BLOOD SERUM FROM VARIOUS PATHOLOGICAL CONDITIONS ON HUMAN PLACENTA

As the final step in the investigation a series of experiments was run on dogs in various pathological conditions in order to determine whether these involve an increase in the non-specific serum enzymes. The results are given in Table VI. In conditions I and III the dogs tested negative before operation. The increase in ferment activity

TABLE VI

Action of blood serum from various pathological conditions on human placenta

CONDITION	NUMBER OF TESTS	RESULT
I. Cachexia, pancreatic diabetes.....	4	All of the tests were positive at one stage or another
II. Acute distemper.....	2	
III. Jaundice.....	7	
IV. Pneumonia.....	1	
V. Ulcer of stomach and distemper.....	2	
VI. Generalized infection.....	1	

was clearly evident in these animals. When the loss of weight began to be noticeable there was an increase in the enzyme strength as shown by the decreased time of incubation necessary to give a positive test. When this loss in weight became very rapid the test was always given within the specified time of the Abderhalden reaction.

DISCUSSION

It would seem that the data presented in Tables I, II, and III can be explained on either of these three hypotheses (a) a specific enzyme capable of acting upon placental protein matter; (b) an increased concentration of non-specific enzymes which digest placental protein; plus the action of specific enzymes; or (c) enzymes which act upon the proteins of the serum and yield dialyzable products. *Regardless of the mode of action, there is a definite increase in the proteolytic ferment strength of the blood serum during pregnancy.* This condition is not limited to human pregnants but is also found in pregnant dogs, goats, and in other animals (16). As mentioned before, Abderhalden believes that this increase in ferment activity is of a specific character and is developed in response to a specific protein antigen. He assumes that because of this specific character the enzyme acts only upon placental proteins or proteins of very similar structure. There is nothing in our results to substantiate this view. That is to say, the increased proteolytic power of pregnancy serum may be due to other factors.

In Table IV we have presented results which show that the Abderhalden reaction is more intricate than at first supposed. The results observed here were so constant as not to be due to errors in technic. It will be noted that the kidney was refractory to the action of blood serum from pregnant individuals. This refractory character we cannot explain at present. It is not attributable to the use of one preparation, as, new preparations were made several times and the same refractory character seen. It is clear, therefore, that in pregnant as well as in non-pregnant mammals there exists one or more non-specific enzymes or a number of specific enzymes capable of acting upon protein tissue substrates and producing dialyzable products. Similar results have been obtained by previous investigators. In the Abderhalden test we are dealing either with a production of new, non-specific enzymes or an increase in the power of enzymes already present. It is very likely that a highly specific enzyme does exist in the blood of pregnants, but if so, the dialysis method as developed now and the

Williams and Pearce method do not serve to separate its action from the action of the nonspecific enzymes which are certainly present. In other words the coagulation method does not afford us a means of procuring a proper substrate for the supposed specific enzyme to confine its action to. Furthermore, on the basis of Table V, it is most reasonable to suppose that what we are dealing with is an augmentation of the digesting powers of enzymes already present in the blood preceding the period of gestation; i.e., that we have a quantitative increase. This increase in the action of the non-specific enzymes *may* be further augmented by the presence of a specific enzyme. In Table V it is clearly shown that non-specific enzymes do exist in normal blood serum. This is not a new fact, but a fact in some instances denied, and in no instance sufficiently emphasized by Abderhalden and his pupils.

In 1904 Hedin (17) showed that the blood serum of a normal ox has a weakly proteolytic action on casein and gelatin in alkaline solution. The ferment or enzyme was held apparently in the globulin fraction. At the same time Delezenne and Pozerski (18) brought forward evidence to show that blood serum incubated under chloroform gained in proteolytic power. More recently Kjaergaard has shown this proteolytic power of normal blood serum. The conception has been clearly stated most recently by Vaughn (19).

In a communication to the Chicago Pathological Society (article not yet published), Falls has given further evidence that during digestion the ferment power of the serum of the portal blood is greatly increased when compared with that of the peripheral blood (femoral). If the portal blood does show this enzyme increase during digestion it is highly probable that some of these are present in the normal serum at all times.

Our method for the detection of these enzymes was simply extending the time of the incubation. Some of the tests were so arranged that the final step was performed at the same time for twenty and forty-four hour samples. The marked difference in the reactions left no doubt as to the greater degree of dialysis and consequently the greater amount of digestion in the specimen incubated more than twenty hours. It will be noted that in no case was a positive reaction obtained in twenty hours upon normal serum whereas in only two cases was the test doubtful at the end of forty-four hours. Of the other tissues used kidney was again refractory. The tests are not explicable on the basis of fetal remnants since males were used. In the application of the method

to normal blood it was found that in practically every case there was a reaction in forty-four hours. In a few cases the reaction was apparent in thirty-six hours but rarely before. From this fact we feel safe in assuming that the differentiation of *normal* and pregnant blood can be made on the basis of the present test using the specified time of sixteen to twenty hours as the period of incubation and if the test is positive the individual is pregnant or is subject to some pathological condition.

A view recently presented by Jobling, Eggstein and Petersen (loc. cit.) assigns the mode of action of the ferments in pregnancy to an adsorption of antitrypsin which permits the auto-digestion of the proteins of the serum. Bronfenbrenner believes that the action is a sensitization of placenta by the serum. If the reaction is purely one of adsorption we should have expected positive tests with kidney as substrate. Some believe the reaction to be a typical antibody production. If so we should expect it to be a specific reaction. In our hands this is not the case.

In this connection the experiments on the goat are interesting. Our results show a considerable decrease in ferment activity even previous to the birth of the kids. Whereas on February 22, 1 cc. of serum was sufficient to produce a positive reaction in four cases, on March 11 the same amount did not give a positive reaction. Neither did this amount give a positive test on March 15 or April 14. The interpretation of this is not clear. It may be that some animals reach a condition even previous to parturition in which they can no longer produce the bodies giving to the serum of pregnancy its proteolytic power. In this condition they would resemble those "animals which under the continuous influence of antigen eventually lose the power to produce antibodies" (Hektoen, Harvey lectures, January 15, 1910).

In October of 1914, Falls (loc. cit.) reported a series of experiments upon the reaction of placenta with serum from certain pathological conditions. He concluded that "in many conditions of altered metabolism . . . . . there is an abnormal amount of ferment in the blood." Others have reported similar results. Recently King (20) has reported an increase in the diastases of the blood during wasting conditions. Our results confirm the work of Falls (Table VI). Since in normal serum we have non-specific ferments present invariably it is reasonable to suppose that in the conditions here cited we are dealing with an increase of these same enzymes, especially if such conditions are induced without the presence of an infectious organism. In the

condition of acute pancreatic diabetes and jaundice we are very definitely dealing with a quantitative increase in enzyme strength.

In the early stages of the condition noted above the differentiation on a time basis between pregnancy was easy since no reaction was given in twenty hours. When the condition had advanced this method was of no avail. We believe, therefore, that this method of differentiation between pregnancy and certain pathological conditions is of little value especially if the pathological processes are far advanced and of a wasting character. In other words, the non-specific enzymes are so increased as to mask the results of a specific enzyme if it be present (as in pregnancy).

We believe that at the present time the Abderhalden method does not provide a reliable test for the differential diagnosis between the strictly physiological state of pregnancy and certain pathological conditions.

CONCLUSIONS

I. There is an increased proteolytic ferment action of the blood serum during pregnancy.

II. This increased activity is probably in the main due to an increase in the polyvalent ferments.

III. Normal blood serum has a weak non-specific proteolytic action.

IV. The dialysis method of Abderhalden or the method of Williams and Pearce does not suffice to demonstrate a ferment developed during pregnancy acting only upon placental protein.

V. Many advanced pathological conditions give a positive reaction within the time specified for the Abderhalden test of pregnancy.

VI. At present the Abderhalden test is a quantitative not a qualitative test.

Thanks are due Dr. Carlson for many helpful suggestions during the course of this work.

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THE OXYGEN AND CARBON DIOXIDE CONTENT OF THE BLOOD DURING HIBERNATION IN THE WOODCHUCK (MARMOTA MONAX)

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INTRODUCTION

Of the several theories attempting an explanation of the cause of hibernation in mammals, a most interesting one, especially in the light of renewed work on the rôle of CO_2 in the dormancy of plants (1), is the theory that so-called winter-sleep is due to the accumulation of CO_2 in the blood and tissues of the animal. This excess of CO_2 is supposed to cause a form of narcosis as seen in the torpid condition of the hibernating animal. When the CO_2 reaches a certain concentration, the respiratory center is excited, respiration accelerated and the muscles become hyper-irritable. These culminating results are responsible for the awakening from dormancy.

A suggestion of this theory is found in the work of Bert (2), who in 1873 concluded that the dormancy may be due to the accumulation of CO_2 rather than a diminished supply of oxygen, as he had earlier believed. It was, however, apparently worked out independently as the *carbonic auto-narcosis* theory by Dubois (3) (1895). It appears that the attention of Dubois was called to the researches of Bert by Gley (4) some time after Dubois had published his carbonic auto-narcosis theory of natural sleep.

Dubois claims (5) that he can induce typical hibernating sleep by causing the active marmot to breathe a mixture of air (43 per cent), CO_2 (45 per cent) and oxygen (12 per cent). Further, he shows that torpid marmots remain dormant if supplied with this mixture; but by increasing the amount of CO_2 such animals may double their rate of respiration in ten minutes and may be awakened apparently as they naturally awake from lethargy. The author maintains (6) that the CO_2 cats principally on a nervous center for sleep and awakening situ-

ated in the mid-brain, since marmots deprived of cerebral hemispheres can sleep and awake, but with the bulb (medulla), only, in tact they are unable to wake up.

In support of the above conception Dubois showed (7) (1894), for the first time, that CO_2 actually accumulates in the blood during hibernation of the marmot and decreases again when the animal wakes up and becomes active. He found, on the other hand, but little difference in the amount of oxygen in the blood during the two states.

Dubois worked with the common European marmot (Marmot vulgaris). The figures given by him show that there is 15.4 cc. to 18.06 cc. of oxygen per 100 cc. of arterial blood after two to ten days of torpidity. In the normal active animal he found 15.3 cc. of oxygen in the arterial blood. In the venous blood there is only 6.05 cc. of oxygen after ten days of lethargy, as compared with 8.75 cc. during activity. The quantity of CO_2 is always greater in the blood of the marmot than in the blood of the rabbit, which was used as a control, the difference being especially great when the marmot is hibernating. In the marmot during winter-sleep there is from 63.23 cc. to 76.16 cc. of CO_2 per 100 cc. of arterial blood after two to ten days of torpor, as compared with 41.33 cc. during the active state. In the venous blood after ten days of torpor there is 74.05 cc. of CO_2, as compared with 52.33 cc. during activity. The nitrogen, which amounted to about 2 cc. in each case, does not concern us here.

In regard to the decrease in CO_2 in the blood upon awakening from lethargy, Dubois (8) gives the average results obtained by the analysis of four samples of arterial blood taken from marmots (the number is not stated) which had awakened naturally during the hibernating period, as follows (in cubic centimeters per 100 cc. of blood): O = 14.35, CO_2 = 52.3, N = 2.8, total gas = 69.45. The corresponding figures for three samples of venous blood are: O = 78, CO_2 = 53.5, N = 2.6, total gas = 63.9. He also gives the following figures as the result of analysis of the blood of two marmots artificially awakened during winter and taken to a warm place where they were fed for eight days: O = 14.55, CO_2 = 57.37, N = 2.7, total gas = 74.62 in arterial blood; and in the venous blood, O = 6.63, CO_2 = 56.1, N = 3, total gas = 65.73. Finally, the figures resulting from the analysis of blood taken from marmots (the number is not stated) in summer after feeding for five weeks, are : O = 15.66, CO_2 = 48.4, N = 1.6, total gas = 65.66 in arterial blood; and in venous blood, O = 11.13, CO_2 = 55.3, N = 2.26, total gas = 68.69.

Dubois further shows that the blood of the torpid marmot has a greater capacity for the absorption of CO_2 and oxygen than has the blood of the active animal, due, he thinks, to the greater specific gravity of the blood during lethargy.

The gases in the above analyses were obtained by means of a mercury pump, and were analysed in a eudiometer tube. Nothing is said about the method of obtaining the blood.

From a rather extensive examination of the literature, these facts obtained by Dubois appear to be all that exist on the subject of blood gas analysis during hibernation. Some general observations on the appearance of the blood during this condition are on record. Saissy (9) (1808) observed that the blood during torpor is reddish brown in the arteries just as it is in the veins. Prunelle (10) (1811) found the arterial blood in the bat brighter red than the venous, but darker than ordinary arterial blood. Marshall Hall (11) (1832) said that the blood becomes venous. On the other hand, Valentin (12) (1865) states that in profoundly dormant marmots the venous blood is nearly as red as the arterial and is a cherry red, reminding one of the blood of reptiles and embryos. Quincke (13) (1882) agrees with this observation. Claude Bernard (14) (1859, 1878) and Marès (15) (1892) similarly state that all the blood is arterial during hibernation. A more recent observation is recorded by Allen Cleghorn (16) (1910), who says that he can confirm the previous observations on the arterial character of the blood, for he finds that it has an arterial or bright red hue in the veins. These conflicting statements give no real information as to the actual gas content of the blood during the two states of the animal. In the woodchucks used in the analyses to be recorded here, there was found to be a very pronounced difference in the color of the arterial and the venous blood during the torpid state. The arterial blood is bright red and the venous blood is dark brown, ordinarily, both during lethargy and during activity. But in animals that are being excited and are waking up, the venous blood may become nearly as bright as the arterial. This may be due to the greatly increased circulation and respiration, and may account for the above observations on the arterial character of the blood during winter-sleep.

PRESENT INVESTIGATION

Since actual gas analysis of the blood of hibernating animals is limited to the few determinations made by Dubois, and since Mosso (17) (1899) has an opposing acapnia theory according to which hibernation is due

to the lack of CO_2 in the system, the present investigation was undertaken to see what conditions in this particular occur in the woodchuck. It is well known that the woodchuck (American marmot) is one of the best examples of a hibernating mammal in this country. All species go into winter-sleep from four to six months each year (18). In this vicinity (Ithaca, New York) they seem to retire, as a rule, by the beginning of November, and they are seen again running about in March. They do not store any food in their burrows. In captivity, at least, they may wake up a few times during the winter. They are occasionally found sufficiently awake to retreat slowly to some other part of the burrow when exposed to the cold air. Most of the time, however, they are very dormant.

The animals used in this study were captured uninjured in the last week of August and first week of September, 1914, in this neighborhood. Six of them were kept in an open dog court at the laboratory in a box, 43 x 30 inches and 30 inches deep, with an extra bottom, 6 inches above the main bottom, so as to raise the animals above the ground. This box is lined with expanded iron and filled with straw. It communicates with a feed box of about the same size, lined with galvanized sheet iron, and covered by a lid of expanded iron. This feed box was removed on November 21, 1914, when the woodchucks commenced to hibernate, and the animals were shut up in the straw-filled box, which in turn was inclosed in a wooden structure, 6 feet 4 inches x 4 feet 6 inches and 4 feet high and covered by a roof of corrugated iron. The space between this enclosure and the box containing the woodchucks, was packed with dry straw. In this manner the animals hibernated very well till the end of February when the last one of this lot was killed. The rectal temperature of these animals between December 5 and February 27 varied from 6°C. to 14°C.

The rest of the woodchucks were kept in three of the eight artificial burrows mentioned by Simpson (19) three years ago. Since few details were given at that time, the opportunity for a more detailed description is taken here.

Each burrow consists of a sheet iron cylinder, 19 inches in diameter, 5 feet deep, closed at the bottom and covered by a removable tin lid on top. It projects 8 inches above the ground. The wall is perforated with $\frac{1}{2}$ inch holes, 6 inches apart. Near the bottom is a hole large enough to admit the end of a 6 inch glazed tile pipe, which communicates with a central pit or basket of expanded iron, 42 inches in diameter. The bottom of this pit is a little lower than the bottom of the burrow,

just described, and the top is open. Covering this surface opening is a gabled roof of corrugated iron, 60 inches x 51 inches. An opening near the surface of the ground permits the animals to enter the pit and the burrows, or to go out to the surface of the ground at will. The eight burrows encircle this central pit, 20 inches distant. The whole is surrounded by a galvanized sheet iron enclosure, 14½ feet x 14½ feet and 6 feet 8 inches high, floored with expanded iron 10 inches below the surface of the ground. This enclosure is covered overhead by heavy wire netting to keep out dogs, etc., since it is located about half a mile from the laboratory on the slope of a small hill. The central pit and the burrows are filled with dry straw.

The torpid animal is always found near the bottom of a burrow in a nest of dry straw. It will be seen that the upper portion of the dormant animal is thus about 48 inches below the surface of the ground. This, apparently, is about the depth at which these animals naturally hibernate, according to the description of the natural burrows given by Fisher (20).

All the animals were given fresh water and food, such as green clover, corn, apples and carrots, every other day till November 19, 1914. From that date no water or food was obtainable by these animals till after hibernation. Feeding was not recommenced till April 10, 1915, on account of the late spring. Woodchucks have hibernated well in these artificial burrows every winter for several years.

METHOD

At various periods preceding, during, and after hibernation, animals were taken and disturbed as little as possible while the blood samples were rapidly drawn. If the animal was active it was rapidly etherized and tied to an animal board. In one case chloretone was used as the anaesthetic in order to check the other results. If the animal was torpid no anaesthetic was used (with one exception to be mentioned later) since it is possible, by keeping the dormant animal cool either by operating in winter near an open window or by packing the animal with snow, to operate for an hour or longer without disturbing it much. The femoral artery was then isolated ready for the insertion of a cannula. The external jugular was next isolated and a small T tube, with the short limbs minimal in length, was inserted near the lower end or base of the vein so as to drain from both directions. In this way it was possible to get blood enough from the vein, notwithstanding the low blood pressure encountered, especially during hibernation. After allowing the blood to wash out the rubber connections attached to the cannula,

from 3 cc. to 4 cc. of venous blood was collected and as soon as possible (within five minutes from the time bleeding was commenced) placed under the ammonia solution in the bottles of the apparatus. Then an ordinary cannula was inserted into the femoral artery and a similar quantity of arterial blood was collected in the same way and immediately placed in the apparatus. The whole operation of getting the blood samples was accomplished as a rule in forty minutes or less.

The analysis was carried out by the chemical method of Haldane (21) with the apparatus devised by Brodie (22), which was calibrated by the method of Hoffmann (23). Five manometers were used for each sample of blood in order to reduce mechanical errors. Due to the small quantity of blood in the animal and the desirability of getting samples both of arterial and of venous blood as rapidly and as near together as practicable, with the least possible disturbance of the animal, it was deemed better to get one large sample of each kind of blood rather than several smaller ones. The average of the five determinations made on each sample was taken as the amount of gas in the blood.

RESULTS

The results and other data relating to the animals, are recorded in the table below.

Animal (Series II)	Date of Analysis	Gas in cc. per 100 cc. of blood reduced to 0°C. and 760 mm. Pressure			Condition of Animal	
		A = Arterial V = Venous D = Difference	O_2	CO_2	Rectal Temperature—C.	Anaesthesia, etc.
3	Sept. 28, 1914	A	17.82		35.8	Active, before hibernation, ether anaesthesia
		V	11.85			
		D	5.97			
4	Oct. 17, 1914	A	24.01	60.69	36.4	Active, before hibernation, ether anaesthesia
		V	19.64	64.28		
		D	4.37	3.59		
5	Nov. 7, 1914	A	22.60	75.06	33.9	Active, before hibernation, ether anaesthesia
		V	13.35	76.31		
		D	9.25	1.25		

ANIMAL (SERIES II)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF BLOOD REDUCED TO 0°C. AND 760 MM. PRESSURE			CONDITION OF ANIMAL	
		A = Arterial V = Venous D = Difference	O_2	CO_2	Rectal Temperature—C.	Anaesthesia, etc.
6	Dec. 5, 1914	A	28.79	87.69	14	Asleep, just commenced to hibernate, ether which woke animal up and greatly excited respiration
		V	18.52	93.20		
		D	10.27	5.51		
7	Dec. 19, 1914	A	25.76	68.80	6	Torpid, no anaesthesia
		V	6.16	96.00		
		D	19.60	27.20		
8	Jan. 16, 1915	A	23.08	43.01	6 but rose to 20	Torpid, no anaesthesia. Woke up before blood was obtained, respiration increased from 11 to 34 per min.
		V	12.93	71.24		
		D	10.15	28.23		
10	Feb. 6, 1915	A	22.20	83.06	6	Torpid, no anaesthesia
		V	6.65	98.08		
		D	15.55	15.02		
11	Feb. 27, 1915	A	20.23	86.39	9	Torpid, no anaesthesia
		V	13.53	100.64		
		D	6.70	14.25		
12	Mar. 20, 1915	A	16.09	76.40	25	Awake but rather sluggish, torpid 6 days previous, ether anaesthesia
		V	11.52	79.18		
		D	4.55	2.78		
13	April 10, 1915	A	12.39	74.61	36	Been awake for about 3 weeks, nothing to eat, chloretone per rectum
		V	7.09	80.56		
		D	5.30	5.95		
14	May 18, 1915	A	14.83	59.30	37.2	Been awake for 2 months, fed for 5 weeks, ether anaesthesia
		V	8.86	60.57		
		D	5.97	1.27		

It will be noted that there is an increase of about one-third in CO_2 during hibernation. This agrees with the results obtained by Dubois on the European marmot. The increase is especially marked in the case of the venous blood, where the CO_2 amounts to over 100 vol. per cent in the latter part of torpidity. The difference between the amount of CO_2 in the venous blood and in the arterial blood reaches its highest at about the middle of the period of torpidity. This great difference may be due to greater respiratory exchange as a result of slow circulation. Upon waking up there is a fall in the CO_2 in the blood as was also noted by Dubois. This fall is especially marked in the venous blood. At all stages included in this period of observation, there is a larger amount of CO_2 than is normally the case in most mammals, a fact also observed by Dubois in case of the European marmot.

In case of animal No. 8 we failed to get the venous blood to flow from the jugular on the left side and hence had to go to the right side, where the operation was also attended with some difficulty. In the meantime the animal woke up before any blood was obtained. During the one hour and twenty minutes consumed in getting blood, respiration increased from 11 to 34 per minute and the rectal temperature rose from 6° C. to 20°C. The increased respiration of the animal necessarily vitiates the results. It appears that the amount of CO_2 in the blood was greatly reduced, especially in the arterial blood. This sudden fall in CO_2 in the blood would contribute to the increased R. Q. observed by many (24) in hibernating animals as they wake up. The oxygen in the venous blood was evidently increased to about twice the amount usually found during torpor in the woodchuck.

Another irregularity in the series is found in connection with animal No. 6. This woodchuck had just become dormant as is evident from the date and the rectal temperature. Ether was given to prevent it from waking up and struggling. The ether greatly excited the animal, increasing the respiratory movements and waking it up while the blood samples were being taken. A high oxygen content but not a low CO_2 content curiously resulted.

The amount of oxygen in the blood varies much more than is indicated by the figures given by Dubois. The results here show a much higher percentage of oxygen, especially in the arterial blood, in the woodchuck during and just preceding winter-sleep (amounting to 26 vol. per cent in animal No. 7) than was found in the European marmot. In view of the very recent work by Christiansen, Douglas and Haldane (25) (1914) on the absorption and disassociation of CO_2 by blood, show-

ing that oxygen tends to drive out CO_2 and that the action depends upon the saturation of the hemoglobin, the large amount of CO_2 in the presence of a high percentage of oxygen indicates other changes in the blood of the woodchuck during hibernation.

Another evident fact is the great difference between the amount of oxygen in the arterial blood and in the venous blood during torpidity. This difference amounts to 19 vol. per cent in animal No. 7. This may also be due to slower circulation and the resulting greater respiratory exchange.

Aside from the bearing these facts may have on the carbonic autonarcosis theory of Dubois, they suggest that the hemoglobin or other components of the blood undergo some marked changes which may either have something to do with the onset of hibernation or are the results of the lethargy. The apparent increase in the oxygen and CO_2 absorbing capacity of the blood during winter-sleep, as was indicated by Dubois (26) and which he attributed to an increase in the specific gravity of the blood (27), needs further investigation because facts which have not yet been published indicate that there is no marked increase in the specific gravity of the blood in the woodchuck during hibernation.

A criticism may be offered from the fact that ether was generally used as an anaesthetic on the active animals, while no anaesthetic was used on the torpid ones, except as was mentioned above in connection with animal No. 6. The ideal condition for this experiment would be to get the blood from the normal animal at all times. The active woodchuck, however, is vicious and must be immobilized. Chloretone, which in general is fairly satisfactory, seems to take effect too slowly when given per rectum even in large doses. It is practically impossible to get the drug into the stomach in this case since the animal can be handled only by suspending it by its tail. The least disturbance of the respiration of the active woodchuck was found to result from placing the animal in a box where the air was already saturated with ether. In no case—except in No. 6, where the animal was really dormant—was there a noticeable increase in respiration due to ether, although ether is generally considered to be a respiratory excitant and tends to increase the amount of oxygen and decrease the amount of CO_2 in the blood (28). The rather low per cent of oxygen in the blood of the active animal would seem to indicate that there was little if any over ventilation. The result in the case of animal No. 13 where chloretone was used (chloretone being considered a depressant), also indicate that but little error has crept in because of the use of ether.

SUMMARY

1. The amount of CO_2 in the blood of the woodchuck is at all times great as compared with that of most of the other mammals whose blood has been analysed.

2. The amount of CO_2 increases progressively during hibernation and decreases again when the animal wakes up.

3. There is also a larger per cent of oxygen in the arterial blood just preceding and during torpidity than at other times.

4. The difference between the amount of CO_2 in arterial and that in the venous blood is much greater during hibernation. There is also generally a greater difference between the amount of oxygen in the arterial and that in the venous blood during the dormant state.

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BLOOD COUNTS IN THE FROG, THE TURTLE AND TWELVE DIFFERENT SPECIES OF MAMMALS

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Since 1854, when the enumeration of the blood corpuscles was first successfully accomplished by Vierordt and Welcker, an enormous number of determinations have been made on the human subject both in health and disease, and the number of erythrocytes per cmm. given by these observers as normally present (5,000,000 for men and 4,500,000 for women) may be taken as approximately correct. In the lower animals, on the other hand, apart from those more immediately related to the veterinary profession, comparatively little attention has been given to the subject, and the following observations, made by us in this laboratory within the last six months, may be of sufficient interest to justify their publication.

The subjects were all healthy normal animals kept in the laboratory animal-house and yard, or lent by a farmer in this vicinity for the occasion. As a rule, no anaesthetic was used, but in some cases (badger, woodchuck), immobilization was found to be necessary and then ether was given.

Both red and white corpuscles were enumerated but no differential counts of the whites were attempted. The Thoma-Zeiss haemocytometer was employed throughout with Hayem's solution as the diluting fluid for the reds (dilution 1 in 200) and $\frac{1}{3}$ per cent acetic acid, tinted with methyl violet, for the whites (dilution 1 in 10). One hundred squares were counted and in some cases one hundred and fifty, two hundred and four hundred.

The blood was usually obtained from a slit or puncture wound through the skin of the ear or inner side of thigh, previously shaved, cleaned and dried, and from the drop or pool that collected we each took a sample practically simultaneously. After diluting for the reds and mixing thoroughly, two other samples were taken, in the same way, for the

TABLE

ANIMAL	RED	OBVS.	WHITE	OBVS.	DATE	REMARKS
Dog I ♀	3,697,000	5	12,300	3	Mar. 2	5 days before parturition
Dog I ♀	4,495,000	7	10,580	3	Mar. 4	3 days before parturition
Dog I ♀	3,466,000	4	12,370	3	Mar. 9	2 days after parturition
Dog I ♀	4,912,000	2			Mar. 23	16 days after parturition
Dog I ♀	7,052,000	2	11,000	2	Aug. 5	151 days after parturition
Pup I	4,080,000	2	12,160	1	Aug. 10	3 days old
Pup II	4,777,680	2			Aug. 11	4 days old
Pup III	4,492,800	2			Aug. 11	4 days old
Pup IV	4,095,100	3	11,900	1	Aug. 11	4 days old
Pup V	3,334,900	2			Aug. 23	16 days old
Dog II ♂	6,597,000	4			Aug. 16	2 years old
Dog II ♂	6,710,400	2			Aug. 23	
Dog III ♀	5,662,400	2			Aug. 16	41 days before parturition
Dog III ♀	6,501,000	4			Aug. 23	34 days before parturition
Dog III ♀	6,593,600	2			Aug. 23	Resting
Dog III ♀	7,132,000	2			Aug. 23	Exercise 30 minutes.
Pup A ♂	5,176,000	2	22,800	1	May 1	4 days old
Pup B ♂	3,464,000	2	15,400	2	May 4	7 days old
Pup C ♂	4,872,000	2	19,200	2	May 4	7 days old
Dog IV ♂	6,951,400	2			Mar. 16	2 years old
Dog V ♀	6,716,000	2			Mar. 18	Not pregnant
Dog VI ♀	7,600,000	2			Mar. 18	Not pregnant
Cat I	9,628,000	2	9,000	1	Apr. 13	Adult
Cat II	9,664,000	3	20,600	2	May 6	Adult
Rabbit I ♀	6,344,000	2	18,000	1	Apr. 13	Adult
Rabbit II ♂	7,256,700	2	7,200	2	Apr. 20	Adult
Rabbit III			10,030	10	Apr. 22	Adult
Horse I ♂	7,580,000	2			Apr. 22	Adult gelding
Horse II ♂	8,208,000	2	8,600	2	Apr. 22	Adult gelding
Cow I	7,812,000	2			Apr. 22	Adult
Cow II	7,498,700	3	11,600	2	May 4	Adult
Sheep I ♀	10,994,000	2	5,200	2	May 6	Adult
Sheep II ♀	9,808,000	1	8,800	2	May 11	Adult
Sheep III ♀	10,260,000	2	11,600	1	May 11	Adult
Goat I ♀	11,748,000	2	6,600	2	June 17	Angora; suckling kid
Goat I ♀	11,220,000	2			July 21	Angora milch

TABLE—Continued

ANIMAL	RED	OBS.	WHITE	OBS.	DATE	REMARKS
Goat II ♀	19,760,000	2	8,000	2	June 17	Angora; suckling kid
Goat II ♀	16,970,000	2			July 21	Angora milch
Kid I ♂	19,376,000	2	12,250	2	June 18	76 days old
Kid II ♂	22,344,000	2	12,800	2	June 18	69 days old
Swine I ♀	8,120,000	2	12,500	2	May 11	Adult
Swine II ♀	7,600,000	2	10,600	3	May 13	Adult
Monkey ♀	6,212,000	2	5,200	2	June 18	Adult
Prairie-dog I ♂	9,901,760	2	6,400	2	Mar. 30	Adult
Prairie-dog I ♂	8,057,600	3	6,200	3	Apr. 10	11 days after operation
Prairie-dog II ♂	9,926,900	2			Mar. 30	Adult
Prairie dog II ♂	8,201,400	2	9,200	2	Apr. 13	14 days after operation
Prairie-dog III ♂	9,694,000	2	6,400	1	Mar. 30	Adult
Prairie-dog III ♂	8,452,600	2	7,800	2	Apr. 13	14 days after operation
Woodchuck I	7,748,000	2	14,000	1	July 8	Adult
Woodchuck II ♀	6,097,000	6	10,300	2	Aug. 5	Adult
Woodchuck III ♂	6,296,000	2	12,400	1	Aug. 5	Adult
Badger I ♀	13,777,400	4	15,710	2	Mar. 25	2 days before parturition
Badger I ♀	14,213,000	2	16,730	3	May 20	54 days after parturition
Badger II ♀	7,880,000	2	14,100	2	June 18	83 days old
Badger II ♀	11,440,000	2	10,650	2	Aug. 5	132 days old
Tortoise I	388,000	1	12,200	1	July 6	
Tortoise II	896,000	1	13,600	1	July 6	
Tortoise III	984,000	1	11,200	1	July 6	
Frog I	652,000	5			Apr. 22	
Frog II	736,000	1			Apr. 22	
Frog III	616,000	1			Apr. 22	
Frog IV	360,000	1	10,400	1	May 8	

whites, and set aside in the counting chamber until the red corpuscles had been enumerated, four haemocytometers being used for each set of observations. Previous to this work we had had considerable practice in blood counting and as a rule our independent enumerations did not differ by more than 2 per cent, frequently by much less; when the disagreement was serious both were discarded and fresh samples taken.

Our results are presented in the preceding table where the average numbers of red and white corpuscles are given for each set of observa-

tions and the dates on which these were made; e.g., in dog 1, on March 2, the average of 5 counts was 3,697,000 for the reds, and of 3 counts 12,300 for the whites, etc.

Dog I, 7 years old, gave birth to her eighth litter of pups on March 7. The counts just before and after parturition show an unusually low number of red cells which appear to increase as the puerperium advances. With regard to the effect of pregnancy and parturition on the red cells opinions differ. Cohnstein (1) found an average of 9,742,000 in 7 pregnant and 12,090,000 in 12 non-pregnant sheep. From the examination by Thompson (2) of 12 pregnant women at different stages of gestation, he concludes that there is a moderate increase in red cells early in pregnancy, a diminution in the middle months with an increase again to the normal number towards its termination. Burnett and Traum (3) found that the count remained low in the bitch for some weeks after parturition, and a similar statement is made regarding the human subject.

The effect of age on the number of corpuscles is interesting. It is generally stated that the number of red cells is greater in the newly born and less in adolescents than in adults, but the evidence for this statement is conflicting. In the young pups of both dog I and dog III (see table) the red cells are distinctly below the average number for the adult normal dog, whereas, in the kids of our two goats the conditions are reversed. The figure 22,344,000 obtained in kid II is a higher count for reds than we have been able to find recorded anywhere in the literature, for any species of animal. Storch (4), on the other hand, found the average number of reds in adult goats to be 14,569,000 and in kids 10,150,000; in adult sheep (ewes) 9,039,000, in lambs (1–14 days old) 8,833,000, and in lambs two months old 13,232,000; in adult horses 7,639,000 and in foals 9,340,000; in adult cattle 6,219,000 and in calves 8,523,000; in adult swine 8,045,000 and in pigs 4,923,000. According to Burnett and Traum (3) the average number for dogs is 5,967,950 per cmm., while puppies, from less than a day to 20 days old, have 3,992,000 to 4,134,000 per cmm. Hayem (5) gives the average for cats as 9,900,000 while for kittens, from 4 to 8 days old, it is 5,357,000.

The badger on which we made our observations was a fine healthy specimen obtained from the state of Kansas. Two days after the first series of counts was made it gave birth to a litter of one, a female. This last we succeeded in rearing and domesticating and on June 18, when it was about three months old, the red cells numbered 7,880,000

per cmm. as compared with 14,213,000 for the mother a month before; on August 5, however, they had increased to 11,440,000.

From the above records it will be seen that no general statement can be made, covering all animals, regarding the effect of age on the number of red cells found in the blood.

The number of individuals of each species examined by us is not sufficient on which to base any conclusions concerning the influence of sex on the number of red corpuscles present in the blood.

The effect of muscular work is seen in the case of dog III (see table) where thirty minutes' exercise, running up and downstairs, raised the count from 6,593,000 to 7,132,000.

The three prairie-dogs were used in the laboratory for brain experimentation, the blood being obtained from the extirpation wound made in the cerebral cortex when the skull was trephined, and again when the animals were killed, about a fortnight after the first operation. In every case the red count is distinctly lower after than before the operation.

We find, for all the species examined, that there is much greater variation, relatively, in the number of white corpuscles than in the number of reds, both amongst different individuals of the same species and in the same individual from time to time. In forty-seven ordinary street dogs, such as find their way into a laboratory, and judged from their general condition to be normal, Musser and Krumbhaar (6) found that the average number of red cells was 5,973,739 per cmm., the highest count being 7,760,000 and the lowest 4,630,000. The white corpuscles were enumerated in only twenty-four of these animals, the average count being 15,923 and the extremes 33,050 and 8,800. All our dogs, with the exception of No. 1, had been born and bred in the animal house attached to the laboratory and were about two years old at the time the blood was examined. For these six dogs, three males and three females (excluding the counts taken from No. I near parturition, and from No. III as the result of exercise), the average for the red cells was 6,709,300 with extremes 7,600,000 and 5,662,400.

SUMMARY

From 229 blood counts made on 48 animals of 14 different species the average number of red and white blood corpuscles, respectively, per cmm. for each species was found to be as follows: Dog, adult, red 6,709,300, white 11,000; dog (few days old), red 4,268,560, white

16,290; cat, 9,646,000 and 14,800; rabbit, 6,800,850 and 11,743; horse, 7,894,000 and 8,600; cow, 7,655,350 and 11,600; sheep, 10,354,000 and 8,533; goat (adult, angora), 14,974,500 and 7,300; goat (kid, angora), 20,860,000 and 12,525; swine, 7,860,000 and 11,550; monkey (Cercopithecus callitrichus), 6,212,000 and 5,200; prairie-dog (Cynomys ludovicianus (Ord.)), 9,840,880 and 6,400; woodchuck (Marmotta monax), 6,713,700 and 12,250; badger (Taxidea taxus (Schreber)) adult, 13,995,200 and 16,220; badger, three months old, 7,880,000 and 14,100; same badger, four and a half months old 11,440,000 and 10,650; turtle (Chrysemys elegans) 756,000 and 12,330; frog (Rana esculanta), 591,000 and 10,400.

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(1) Cohnstein, J.: Arch. f. gesammt. Physiol., 1884, xxxiv, 233.
(2) Thompson, W. L.: Bull. Johns Hopkins Hosp., 1904, xv, 205.
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(4) Storch, A.: Untersuchungen über den blutkörperchengehalt des Blutes der Landwirtschaften Haussängetiere. Inaug. Diss. Bern; 1901. (Reference from Burnett, "The clinical pathology of the blood of domesticated animals," Ithaca, N. Y., Taylor and Carpenter, 1908, p. 65).
(5) Hayem: Ref. from Burnett: Loc. cit., p. 58.
(6) Musser and Krumbhaar: Folia Haematologica, 1914, xviii, 576.

THE INFLUENCE OF OIL OF CHENOPODIUM ON INTESTINAL CONTRACTILITY

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The present communication is one of a series of studies on the pharmacology of oil of chenopodium which has been carried on in this laboratory at various times during recent years. Some of the results, already reported by the senior author and his collaborators[1,2] indicate that it has toxic properties affecting various tissues and organs of the body. It was of interest, therefore, to inquire in what manner the intestines react in the presence of this substance since, as far as we know, this has never before been the subject of investigation. Moreover, the importance of a better understanding of its action on the intestine is emphasized by its extensive use in the therapeutics of ascarides and hookworm, for any modification in the motor functions of the gut is likely to influence absorption and thus affect the toxicity of the oil. This is especially important on account of the change in the intestinal mucosa produced by the parasites against which oil of chenopodium is employed. (See Osler's *Practice of Medicine.*)

The method introduced by Magnus[3] and adopted later by a number of investigators was used in these experiments. The animals were deeply anesthetized with urethane, the abdominal viscera exposed and segments of equal length, usually about 1.5 to 4 cm. were removed from various parts of the intestines. One end was attached to a glass hook fixed in the center of a rubber stopper, fitting tightly in the lower end of a cylinder of 100 cc. capacity. The latter was filled with Locke's solution, through which was passed a continuous stream of oxygen. The other end of the segment was attached to a recording lever. Exposure and excessive handling of the gut was avoided as much as

[1] Salant and Nelsôn: This Journal, 1915, xxxvi, 440.

[2] Salant and Livingston: This Journal, 1915, xxxviii, 67.

[3] Magnus: Arch. f. gesammt. Physiol., 1904, cii, 123.

possible, the marked irritability and irregular action following such treatment furnishing the indication for this precaution. The cylinders containing the isolated pieces of intestine were kept in a warm water-bath maintained at a uniform temperature by means of an electric bulb. The substances to be tested were kept at the same temperature as the fluid surrounding the intestinal segments and were added to the contents of the cylinders. This procedure was adopted in order to avoid changes of temperature and exposure of the tissues.

A preliminary period during which observation on the behavior of the intestine in Locke's solution was studied preceded the experimental period when the intestine was in contact with the substance under consideration. This was followed by an after period when Locke's solution alone was substituted for the one containing the drug, care being taken to drain this off previously and wash the cylinder a number of times with Locke's solution.

Experiments with oil of chenopodium were also carried out on the intact animal. Rabbits were placed on the holder under urethane anesthesia, the abdomen was shaved and the peristaltic waves recorded by means of hooks[4] attached to the skin from which threads passed to recording levers. The oil of chenopodium was made up in the form of an emulsion with acacia and added to Locke's solution.

EXPERIMENTS ON RABBITS

When segments of the duodenum were placed in Locke's solution containing oil of chenopodium in the proportion of 1 : 10,000, or 1 : 5000, decreased frequency of rhythmic contractions was observed within one or two minutes. Later the amplitude also began to diminish, the decrease being progressive, contractility disappearing altogether in seven and one-half to fifteen minutes after exposure to the influence of the oil of chenopodium. In one experiment with 1 : 5000 oil of chenopodium, this occurred after four and a half minutes; in two others with 1 : 10,000 oil of chenopodium, contractility was but moderately decreased after being subjected to its influence for twenty minutes.

That the injury is apparently not permanent is shown by the fact that complete recovery may take place when the tissues are thoroughly washed with Locke's solution and allowed to remain in it for some time. Coincidentally with the decrease in force and sometimes also in fre-

[4] We are indebted to Dr. Livingston of this laboratory for suggesting this method.

quency of the rhythmic contractions, there was a depression of tone which was sometimes gradual but not infrequently quite abrupt and very marked as shown in figures 1 and 2.

The jejunum seemed to be less resistant to the influence of oil in some experiments, though no marked differences in action were observed in most instances. The decrease of amplitude and depression of tone occurred in two and a half to five minutes, while complete disappearance of contractions took place in seven to fifteen minutes. When the intestinal segments were thoroughly washed, after being subjected to the influence of oil of chenopodium thirty to forty-five minutes and allowed to remain in Locke's solution alone, the rhythmic contractions became normal or improved considerably. A return and sometimes a rise of tone above normal was also observed during this period.

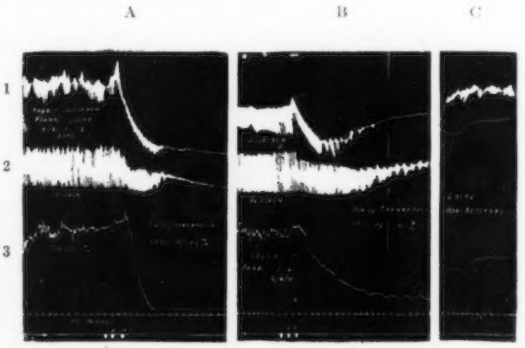

Fig. 1. Rabbit 1742. 1, Duodenum; 2, jejunum and 3, colon (A) subjected to oil of chenopodium 1/5,000. (B) Recovery in Locke solution and effect of 1/10,000 oil of chenopodium. (C) Returned to Locke solution alone

The activity of the ileum placed in 1 : 5000 oil of chenopodium in Locke's solution continued for a variable time. The effect was slight at first, the amplitude as well as frequency being affected. At the end of fifteen to thirty-five minutes, contractility usually disappeared altogether. The tone was also depressed at this time but it was preceded, in some cases, by a primary rise soon after the oil of chenopodium was added to Locke's solution. Only slight improvement in contractility was observed when pure Locke's solution was substituted for oil of chenopodium after the usual treatment. The tone was frequently increased, and was in some cases even greater than in the fore period.

Segments of the colon suspended in Locke's solution containing oil of chenopodium in the proportion of 1 : 5000 manifested depression within one and one-half to four and one-half minutes after exposure. In

some cases complete paralysis was observed immediately after it was subjected to the influence of the oil; in others this was delayed as long as fifteen minutes but in most instances this occurred in two and one-half to five minutes. Although the effects were permanent in a majority of the experiments, contractility with a return of the normal tone might occur when the segments are placed in Locke's solution alone.

Tests were also carried out with 1 : 10,000 oil of chenopodium in Locke's solution. This had no effect on the contractility or tone in some cases, but depression was marked in a good many experiments.

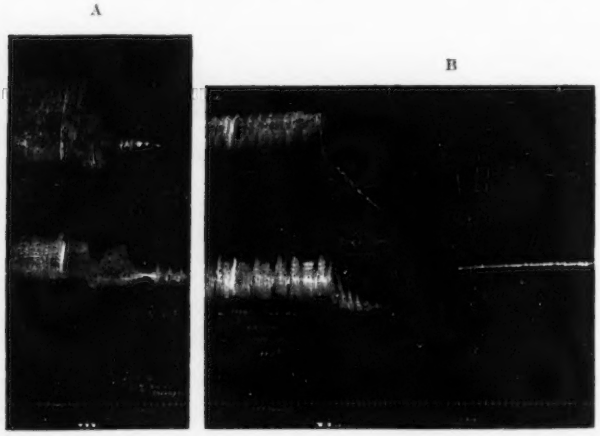

Fig. 2. Rabbit 1765. (A) 1, Jejunum; 2, ileum and 3, colon subjected to oil of chenopodium 1/5,000. (B) Recovery of oleum and jejunum in Locke solution showing effect of second treatment of oil of chenopodium 1/5,000 and recovery when returned to Locke.

It is worthy of remark that when the tissues had been previously subjected to the influence of a 1 : 5000 of the oil, depression of tone was complete, and nearly complete disappearance of rhythmic movements were always observed, even with 1 : 10,000 oil of chenopodium. Partial return of tone but no rhythmic contractions could be noticed when the segments of the gut were returned to Locke's solution alone, thus indicating cumulative effect.

EXPERIMENTS ON CARNIVORA

The response to oil of chenopodium was studied on the intestines of cats and one dog. As the isolated intestine of these animals is frequently apt to be less regular and more sluggish than that of the rabbit, the tests with oil of chenopodium were not quite as satisfactory. Its essential action could nevertheless be demonstrated also in these experiments. This is well shown in figures 3 and 4, and in the following abbreviated protocols which are typical of the results we obtained in a large number of experiments. It will be noticed that in some of

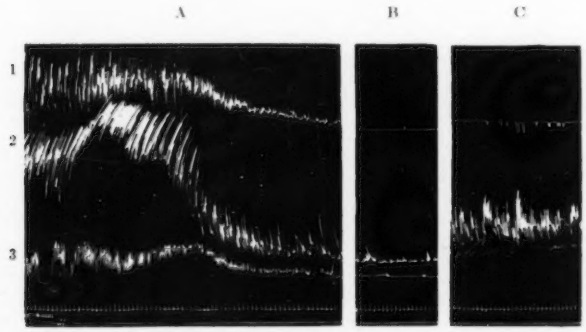

Fig. 3. Cat 394. 1, Duodenum; 2, jejunum and 3, ileum (A) subjected to oil of chenopodium 1/5,000. Curves show primary rise of tone followed by fall together with decrease of pendulum movements. (B) Shows disappearance of contractions in duodenum, weakness of contractions of jejunum and almost no contractions of the ileum. (C) Locke solution alone, showing slight recovery of duodenum and ileum with considerable improvement of the jejunum.

these experiments the influence of pilocarpine and barium chloride on the action of oil of chenopodium were also studied.

Cat 356. Segments of the duodenum, jejunum, and ileum were placed in oil of chenopodium 1 : 5000 for twenty minutes. Increased tone and frequency of pendulum contractions were the initial effects observed in all three portions of the intestine, the increase of tone being greatest in the ileum. Within six minutes depression of duodenal tone occurred and pendulum movements greatly decreased in force and frequency, disappearing altogether sixteen minutes after injection. Contractions and tone of jejunum were paralyzed. Pendulum movements of ileum became feeble and infrequent and disappeared in fifteen minutes, tone falling steadily during the time it was exposed to oil of chenopodium. No recovery of duodenum or of jujenum occurred in pure Locke's solution.

Cat 392. 1 : 10,000 oil of chenopodium in Locke's solution produced stimulation of pendulum movements of duodenal and jejunal segments and depression of pendulum movements of ileum, but the tone was increased. When the concentration of the oil of chenopodium was doubled, depression in all three segments was observed. Complete paralysis of pendulum movements as well as of tone followed with the increase of the concentration to 1 : 3333. Neither $BaCl_2$ 1 : 500 nor 1 : 100,000 pilocarpine hydrochloride had any effect. No recovery was observed when pure Locke's solution was substituted for one containing oil of chenopodium.

Cat 390. Duodenum, jejunum and ileum placed in Locke's solution. Contractions strong. Pilocarpine hydrochloride was added, enough to make a con-

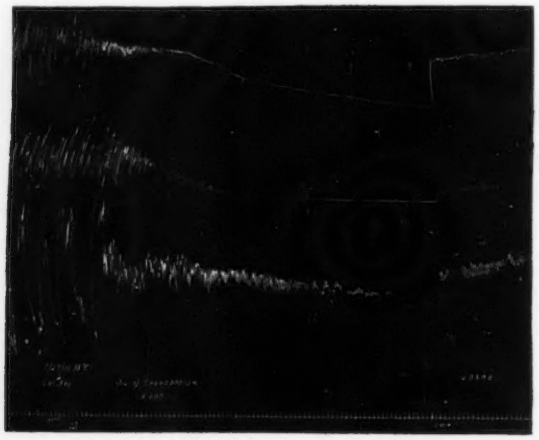

Fig. 4. Cat 395. Action of 1/5,000 oil of chenopodium upon longitudinal strips of 1, Duodenum; 2, jejunum and 3, ileum after cutting circular muscles. Gradual depression of muscular action until contractions disappeared. Partial recovery in Locke solution alone also shown.

centration of 1 : 100,000 caused powerful stimulation. Seven minutes later, 10 cc. 0.1 per cent oil of chenopodium was added, sufficient to make a concentration of 1: 10,000. Contractions began to diminish appreciably five minutes later. Within fifteen minutes after adding oil of chenopodium, contractility of jejunum ceased. Duodenal segment and that of ileum were still active but contractions were feeble. Tone remained almost unchanged in all three segments. Barium chloride added, enough fo make up a solution of 1 : 1000, slightly increased the tone of the duodenum and ileum and lowered somewhat that of the jejunum. Pendulum movements completely disappeared under its influence.

Cat 394. Oil of chenopodium in Locke's solution 1 : 5000. A steady decrease of tone reaching a maximum at the end of thirty minutes was observed. Rhyth-

mic contractions increased in frequency at first, disappearing completely about one hour after being in contact with oil of chenopodium in duodenum and ileum while they were still distinct though feeble in the jejunum. When changed to Locke's solution without chenopodium, contractility returned in the jejunum, the amplitude and frequency being nearly normal. Only slight improvement was observed in duodenum and ileum.

Dog 228. Duodenum, jejunum and ileum in 1 : 5000 oil of chenopodium in Locke's solution. Increase of tone set in immediately after oil of chenopodium was added and continued to rise as long as the segments remained in the oil, which was twenty minutes. After a preliminary increase of the pendulum movements there was a steady decrease which disappeared altogether in the duodenal segments in five minutes. Jejunum and ileum became barely perceptible at this time and continued without change as long as it remained in contact with the oil. When Locke's solution alone was substituted for Locke chenopodium, contractility of duodenal and jejunal segments almost completely revived.

THE INFLUENCE OF DRUGS ON THE ACTION OF OIL OF CHENOPODIUM

Experiments on rabbits. Barium chloride, pilocarpine hydrochloride, and caffeine, all of which are powerful stimulants, were tested for their antagonistic effect on oil of chenopodium. In some experiments they were added to Locke's solution before in others after, the administration of the oil. As shown in figure 6, barium chloride in a concentration of 1 : 3333 added before oil of chenopodium had little effect on its subsequent action. The tone was decreased and the rhythmic movements ceased soon after the intestinal segments came in contact with the oil. It may be remarked that although the decrease in tone was very pronounced, it was not below the normal height in the jejunum and ileum while it was distinctly greater in the duodenum than in the fore period. The reaction to barium chloride in the presence of oil of chenopodium, although considerably diminished, was nevertheless quite distinct when the concentration of the oil was in the proportion of 1 : 10,000. The rise of tone

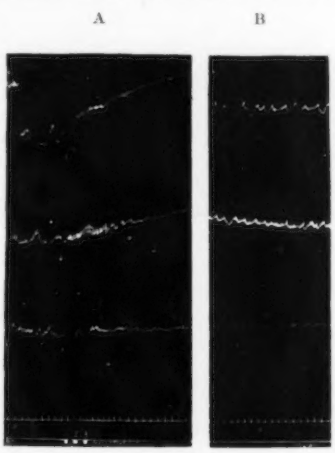

Fig. 5. Dog 228. 1, Duodenum; 2, jejunum; 3, ileum. (A) Showing effect of oil of chenopodium 1/5,000. (B) Recovery in Locke solution without oil.

was abrupt, remaining permanent in some cases but in most instances a gradual descent occurred. In the presence of 1 : 5000 oil of chenopodium, the same concentration of barium chloride (1 : 1000) failed to produce any appreciable rise of tone. The reaction likewise varied with the length of time during which it had been subjected to the influence of the oil previous to treatment with barium chloride. While the increase of tone was quite marked, when barium chloride was added ten to fourteen minutes after the intestinal segments had been under the influence of oil of chenopodium, a longer period of contact with it—thirty-five to forty-five minutes, produced a much weaker response to barium chloride.

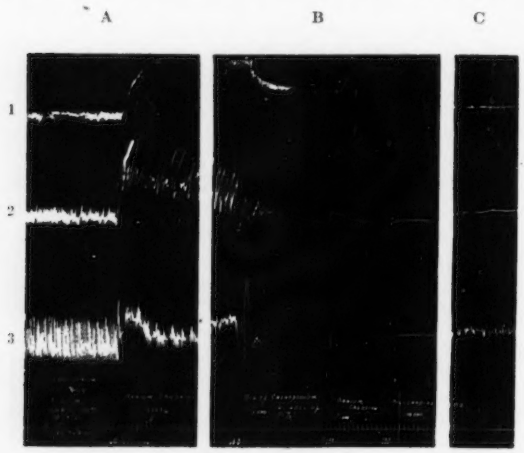

Fig. 6. Rabbit 1872. 1, Duodenum; 2, jejunum and 3, ileum. (A) Barium chloride 1/3,333. (B) Followed by oil of chenopodium 1/5,000. Barium chloride 1/1,000 caused rise of tone but slight reaction to pilocarpine 1/100,000. (C) Returned to Locke solution alone. Slight recovery of duodenum and ileum. No change in jejunum.

The reaction to pilocarpine in various concentrations was usually negative, though in one instance the tone was increased. It may be added that barium chloride and pilocarpine were tried in the same experiment. It was found that the barium effect could be elicited when the tissues no longer responded to pilocarpine (see figure 6), which was mixed with the solution, either before or after barium chloride was added to Locke's chenopodium.

Caffeine in the proportion of 1 : 5000 and 1 : 2000, produced a very marked stimulation of the contractility of the untreated intestine. The effect was different, however, when employed in the same concentrations in the presence of oil of chenopodium, though only used in the proportion of 1 : 10,000. Transitory increase of tone of the duodenum and depression of tone of the ileum, which was permanent in some cases

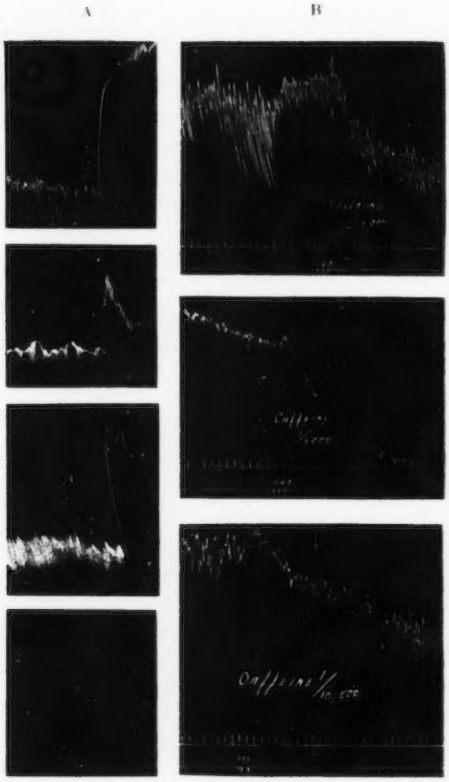

Fig. 7. Effect of 1/5,000 caffeine after subjection of intestine of rabbit to oil of chenopodium 1/10,000. (A) Segments of duodenum. (B) Segments of ileum.

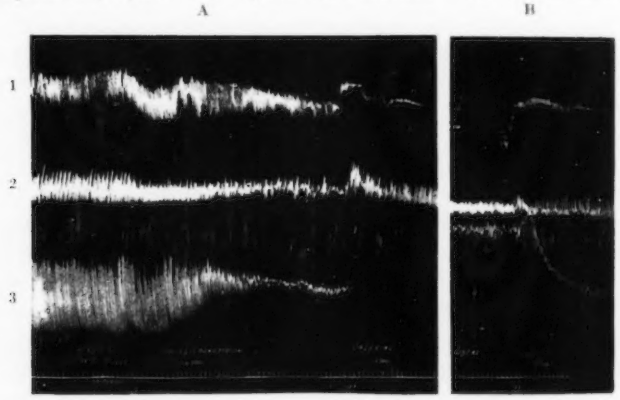

Fig. 8. Rabbit 1868. 1, Duodenum; 2, jejunum and 3, ileum (A) treated with oil of chenopodium 1/10,000 followed by caffeine 1/5,000. (B) shows improvement in Locke solution and effect of 1/5,000 caffeine.

as shown in figure 7, marked this treatment. In some experiments its addition to oil of chenopodium was without any effect. In others, however, this caused occasional stimulation of rhythmic movements. When returned to pure Locke's solution no recovery, or only a moderate degree of activity, could be observed. In one experiment caffeine produced a very pronounced depression of tone in the after period, which suggests that the effect of the oil may persist even after the surviving intestine has been thoroughly washed.

EXPERIMENTS ON CATS

The reaction to barium chloride and pilocarpine was likewise profoundly modified in the intestine of the cat by oil of chenopodium. Little or no response was obtained with barium chloride solutions of 1 : 1000 when the intestinal segments have been under the influence of the oil in concentrations of 1 : 3333 and 1 : 5000. A noticeable increase in tone was observed, however, in one instance when the concentration of barium chloride was increased to 1 : 500 in a dilution of 1 : 5000 oil of chenopodium (Cat 389). The test with pilocarpine was entirely negative in one experiment while a temporary stimulation was observed in another in which the concentration of oil of chenopodium present amounted to 1 : 3333. It may be remarked that in this instance barium chloride, which was added after pilocarpine, produced a very good reaction in the ileum, none in the jejunum, and a mild transitory effect only in the duodenum.

INFLUENCE OF OIL OF CHENOPODIUM ON PERISTALSIS

The movements of the intestines under the influence of oil of chenopodium were also studied in the intact animal. Rabbits under urethane anesthesia were employed for this purpose. This method was found to be particularly well adapted for the study of peristalsis in the living animal since the waves of contractions, especially those of the caecum, appeared in most animals with remarkable distinctness and regularity. A 2 per cent emulsion of oil of chenopodium in olive oil and a small amount of acacia was injected into the ear vein and its effect recorded as stated above. Special attention was paid to size of dose as well as to the influence of repeated dosage. Experiments were also conducted for testing the action of caffeine when given either before or after oil of chenopodium was introduced into the circulation. As a control we used intravenous injections of a solution made up with acacia and cocoanut oil in Locke's solution consisting of the same proportion but without oil of chenopodium. Only a few of these,

however, were made but a much larger number of experiments were carried out with the oil of chenopodium. A detailed description of some of these is contained in the following records, which are typical of the action produced.

April 9, 1915. Rabbit No. 1756, brown and mole color, female. Weight, 1500 Mixed diet. Never before under experimentation. Abdomen shaved.

10.30 a.m. 2 grams of urethane.

2.15 p.m. Peristalsis active.

2.28 p.m. Wave.

2.30 p.m. Wave.

2.33 p.m. Wave.

2.34 p.m. Wave.

2.35 p.m. Wave.

2.48 p.m. 10 cc. of 2 per cent of oil of chenopodium injected into the ear vein. Thirty seconds later the veins of the ear were very much dilated.

3.05 p.m. No true peristalsis though slight pesistaltic waves are seen above the points of attachment.

3.25 p.m. No peristalsis. Respiration shallow with occasional deep breaths. Injection of 80 mgs. of sodium citrate into the ear vein. No peristalsis.

4.00 p.m. No peristalsis. Gut exposed. Oil found in the subcutaneous tissue over the abdomen. Point of contractions mentioned proved to be small intestine.

March 27, 1915. Rabbit No. 1787.

Weight, 1740. Male, Belgian. Mixed diet. Never before under experimentation. Control.

10.00 a.m. 2 grams of urethane.

2.40 p.m. Rapid peristalsis.

2.42 p.m. Injection of 10 cc. of control solution (1 per cent of oil of cocoanut, 0.1 per cent acacia in Locke) in ear vein. No cessation of peristalsis.

2.44 p.m. 10 cc. of control solution in the ear vein.

2.45 p.m. Faint wave.

2.47 p.m. Wave, strong and rapid. Continuous peristalsis.

3.00 p.m. Peristalsis present.

March 27, 1915. Rabbit 1786. Weight, 1310. Male. Black. Mixed diet. Never before under experimentation. Abdomen shaved.

10.00 a.m. 2 grams urethane.

11.00 a.m. Peristalsis active appearing every 40 seconds, strong and rapid.

11.05 a.m. Peristalsis active. Injection of 2.5 cc. of a 2 per cent oil of chenopodium emulsion into the veins of the ear.

11.09 a.m. Three waves passed. Wave in rapid propagation. Waves appear in following periods:

40 seconds.
30 seconds.
40 seconds.
50 seconds.
50 seconds.

11.15 a.m. 7.5 cc. of chenopodium emulsion. Peristalsis depressed. No wave for two and one-half minutes.

11.20 a.m. No peristalsis.

11.30 a.m. No peristalsis.

11.35 a.m. No peristalsis. Upon mechanical stimulation small and slow waves pass over caecum.

11.46 a.m. Faint, slight questionable peristalsis seen at the "Point of Origin" of waves. Perhaps this was the ileum or ileo caecal junction.

12.15 p.m. Slight waves seen.

1.15 p.m. No contractions seen.

2.30 p.m. No contractions seen.

April 2, 1915. Animal weight 1380, apparently normal. Returned to "Runs" and given mixed diet.

March 23, 1915. Rabbit 1712. Weight, 1680. Male. Belgian. Mixed diet. Under experimentation February 9 to March 9.

10.00 a.m. 2 grams of urethane. Abdomen shaved.

3.30 p.m. 4 cc. of 2 per cent oil of chenopodium, 1 per cent oil of cocoanut, and 0.8 per cent acacia in Locke. Injected into ear vein. Peristalsis slightly increased.

3.35 p.m. 5 cc. of the above emulsion given by intravenous injection. Peristalsis decreased in frequency and rate of propagation.

3.46 p.m. 4 cc. of the emulsion injected intravenously. Complete inhibition of peristalsis.

3.56 p.m. Weak contraction just discernible.

4.03 p.m. Mechanical stimulus applied to caecum followed by slight contraction. Repeated stimulation was followed by no contraction.

4.15 p.m. Complete inhibition. Animal placed in isolated cage.

March 27. Normal, returned to "Runs."

April 9, 1915. Rabbit No. 1704. Weight, 1645. Male. Belgian. Mixed diet. Never before under experimentation. Abdomen shaved.

10.45 a.m. 2 grams of urethane.

12.00 m. Peristalsis marked, strong, and rate of propagation rapid.

12.19 p.m. 3 cc. of 2 per cent oil of chenopodium emulsion injected into the ear vein.

11.20 p.m. 4.5 cc. of the above emulsion injected. Followed by depression in the rate of occurrence of peristaltic waves.

12.25 p.m. Three waves since injection.

12.26 p.m. Wave.

12.27 p.m. Wave.

12.28 p.m. Wave.

12.29 p.m. Wave.

12.31 p.m. Wave.

12.33 p.m. Wave.

12.34 p.m. Wave.

12.35 p.m. Wave.

12.39–40 p.m. Injection of 5 cc. of 2 per cent oil of chenopodium. Peristalsis seen in small intestine.

12.42 p.m. No peristalsis in caecum. Some movements in the small intestine, though irregular in frequency.

12.48 p.m. No peristalsis in the caecum; contractions in the small intestine.

1.15 p.m. As yet no peristalsis in the caecum. None seen in the small intestine.

1.20 p.m. No peristalsis, abdomen flat. Animal discarded.

April 12, 1915. Animal up, not depressed and apparently normal. Returned to the "Runs."

DISCUSSION

Whatever the mechanism of the action of oil of chenopodium on the intestine, the results of the experiments presented in this investigation indicate, as in previous studies with this substance, that it is a powerful depressant also of the intestine. The inhibitory effect, however, may be temporary, lasting as long as the tissues are in contact with the oil for if they are thoroughly washed with and then allowed to remain in Locke's solution, recovery varying in degree may occur even after it has been subjected to the action of high concentrations of the oil. The reaction varied in different portions of the intestine, the effect being more marked in the ileum but was much more pronounced in the colon than in the duodenum or jejunum.

That the action is more effective in this case is also shown by the failure of the colon to resume its normal activity when it was surrounded again by pure Locke's solution, as contractility was much weaker or altogether absent.

The work of Weiland[5] is worthy of notice in this connection as it suggests the presence of a substance in the intestine which is antagonistic to oil of chenopodium. It may be recalled that he obtained an extract from the mucosa as well as from the muscular coat which caused powerful stimulation of the movements of the small intestine, but had little or no effect on the large intestine. The failure of this substance to react may account perhaps for the different behavior of the colon from that of the small intestine when treated with oil of chenopodium.

The results on the influence of drugs are of particular interest as regards the mechanism of the action of oil of chenopodium. The stimulating effect of barium, pilocarpine and caffeine was permanently abolished or diminished. In no case did any of these drugs exert any appreciable antagonistic action. Indeed, we observed that caffeine had, on the contrary, a decided tendency to cause sometimes further

[5] Weiland: Arch. f. gesammt. Physiol., 1912, cxlvii, 171.

decrease of tone. Dixon's[6] observations may be recalled in this connection. He found that a strong solution of lactic acid applied externally to the frog's stomach induced contraction followed by gradual relaxation, but when this treatment was employed during the stage of relaxation further depression resulted. The negative results with pilocarpine might indicate that nerve ends as well as muscle fibers are paralyzed, but as stated above, and as shown in figure 6, the response to this drug failed while barium still produced a well marked reaction, thus showing paralysis of the nerve ends. That the muscle fiber is also involved is made apparent by its mode of action in the presence of barium. Although the reaction to barium after subjecting the intestine to the influence of 1 : 10,000 oil of chenopodium was very distinct, it was inferior to that of the normal intestine while it completely disappeared in higher concentrations of the oil. The action of oil of chenopodium is therefore exerted on the nerve ends as well as on the muscular structures, but the evidence brought forward points to the greater resistance of the latter.

The test with oil of chenopodium when injected intravenously may be regarded as corroborative of the results obtained with the isolated intestine. The effect varied with the amounts introduced. The smallest dose given, 0.045 cc. of the oil per kilo produced in one experiment a slight increase of peristalsis, in another case 0.045 cc. per kilo arrested movements of the caecum for four minutes. Medium doses 0.09 to 0.1 cc. per kilo invariably decreased the frequency of peristalsis but total suspension of activity was not noticed after such amounts. This was obtained when the dose exceeded 0.12 cc. per kilo (figure 9), producing in one experiment complete arrest of movements of the caecum for seventy-two minutes. On the other hand, a larger dose produced, in one experiment, decreased frequency only. The data at hand indicate, however, that 0.15 to 0.2 cc. per kilo inhibit peristaltic action in the caecum for a considerable length of time. Only a few observations were made on the small intestine. Well marked contractions were seen after a large dose of oil of chenopodium, while there was complete arrest of movements of the caecum. The large amount of oil of chenopodium required to depress movements in the caecum of the living animal as observed in these experiments deserves explanation, especially since it has been shown by Salant and Livingston[7] that a fall of blood pressure and a marked decrease of the volume of the kidney

[6] Dixon: Journal of Physiology, 1902, xxviii, 57.

[7] Salant and Livingston: l. c.

followed its intravenous administration. That a close relation exists between the blood supply of the intestines and their motor functions has been maintained by a number of investigators. According to vaan Braam Houckgeest,[8] Pal,[9] and others, peristalsis varies with the volume of blood in the vessels of the digestive tract, an increased flow causing stimulation while anemia decreases, or if very severe, may abolish intestinal movements. Paralysis of the vagus, observed by Salant and Livingston, is another factor to be taken into consideration. That it may contribute materially toward the direct action of the oil of chenopodium on the intestine also appears from the results of Meltzer and Auer[10] who found that the elimination of the stimulating effect of the

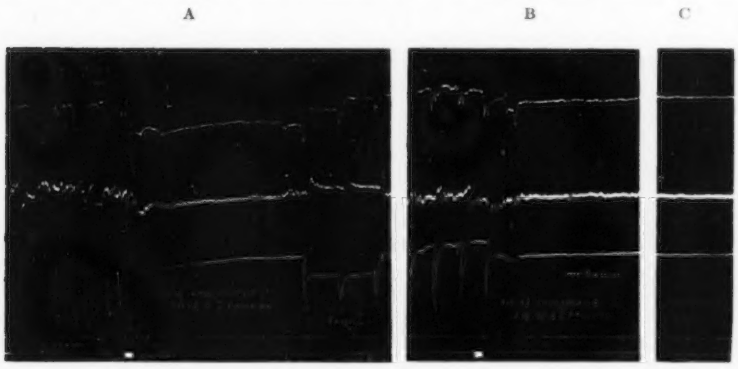

Fig. 9. Rabbit 1777. Peristalsis of caecum in intact animal. 10 cc. of 2 per cent emulsion of oil of chenopodium injected into the ear vein. (A) Movement of caecum after six minutes. (B) 5 cc. more of the emulsion 2 per cent oil of chenopodium injected into the ear vein. No peristalsis followed as seen in (C).

vagus is followed by cessation of caecal peristalsis. Inhibition, partial or complete, of caecal movements also follow, therefore, the introduction of oil of chenopodium into the circulation. Its action on peristalsis when injected intravenously is thus aided, by its effect on the circulation as well as by its depression of the motor nerve of the intestine. That large doses are nevertheless required to produce depression of its activity may be due in the first place to rapid destruction or elimination, but we have seen that this is not probable. There is, on the con-

[8] vaan Braam Houckgeest, Arch. f. gesammt. Physiol., 1872, vi, 266.
[9] Pal: Wiener Med. Presse, 1901, iv, 2017.
[10] Meltzer and Auer: Proc. Soc. Exper. Biol. and Med., 1906, iv, 37.

trary, a marked tendency to cumulation both in the isolated intestine as well as in the intact animal. The explanation may be found perhaps in the presence of neutralizing or antagonistic substances in the tissues of the body. The following observations indeed lend support to this view. Ott[11] stated that the intravenous infusion of an aqueous extract of the spleen stimulates peristalsis while splenectomy is followed by diminished contractility which may be restored to its normal condition or even increased by the extract. The observations of Enriquez and Hallion,[12] and later of Weiland[13] on extracts of spleen and other organs are in agreement with those of Ott. Dixon's[14] findings with pilocarpine in experiments on the frog's stomach are also suggestive in this connection for he noticed that its external application increased the amplitude of contraction waves but had little effect on tone. When injected into the hepatic vein, both tonus and contraction waves were augmented.

In view of the results obtained by Salant and Nelson[15] the relation of the tissue lipoids to the causation of diminished sensitiveness of the intestines deserves consideration since they have shown that the administration of glycerides may decrease the toxicity of oil of chenopodium.

SUMMARY

1. Oil of chenopodium in dilutions of 1 : 5000 and 1 : 10,000 in Locke's solution produces in the isolated intestine of rabbits a marked decrease of tone which remains permanent and diminishes frequency as well as force of contractions which disappeared altogether in twenty to twenty-five minutes. Recovery occurred when the intestinal segments were placed in Locke's solution without oil of chenopodium.

2. In carnivorous animals, oil of chenopodium usually, but not always, causes a preliminary rise of tone followed by a steady decline. Rhythmic contractions may increase in frequency but disappear finally. Recovery may take place when the segments are put into Locke's solution.

3. The reaction to oil of chenopodium was greater in the ileum than in the duodenum or jejunum, but was most marked in the colon.

[11] Ott: Medical Bulletin, 1897, 376.
[12] Enriquez and Hallion: Compt. Rend. Soc. d. Biol., 1911, lxxi, 488.
[13] Weiland: l. c.
[14] Dixon: l. c.
[15] Salant and Nelson: l. c.

4. Caffeine has no antagonistic effect but may, on the contrary, aid depression of tone caused by oil of chenopodium.

5. Neither barium chloride nor pilocarpine has a true antagonistic effect but may prevent to a small extent depression of tone when added before oil of chenopodium. Pilocarpine has no action on intestine which has been poisoned by oil of chenopodium, but barium produces an increase of tone.

6. Nerve ends as well as muscle fiber are attacked by oil of chenopodium, but the latter is more resistant.

7. Relatively large doses of oil of chenopodium are required to inhibit peristalsis in intact rabbits by intravenous injection. The presence of substances antagonistic to oil of chenopodium is offered as an explanation.

THE INFLUENCE OF STIMULATION OF THE DEPRESSOR NERVE UPON SUPRARENAL SECRETION

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During recent years much evidence relating to the secretory innervation of the suprarenal glands has accumulated. Dreyer[1] was the first to show that blood, collected from the suprarenal vein of a dog during stimulation of the splanchnic nerve possessed the power of increasing the blood pressure when injected into the vein of another dog to a greater degree than did blood similarly collected before stimulation. Ample confirmation of this result is to be found in the work of Asher,[2] Tscheboksaroff,[3] Elliott,[4] von Anrep[5] and many others, and it is now regarded as established that secretory fibers for the suprarenal glands are contained in the splanchnic nerves. Elliott's work has further shown that the center of these fibers is situated in the medulla in close association with the vasomotor center.[6] Like the latter, it may be stimulated directly as a result of asphyxia,[7] reflexly by excitation of sensory nerves,[8] and by impulses reaching it from higher centers (mechanical injury,[9] fear, rage,[10] etc.). The nervous mechan-

[1] Dreyer: American Journal of Physiology, 1898–1899, ii, 203.

[2] Asher: Zentralblatt für Physiologie, 1910, xxiv, 927; Zeitschrift für Biologie, 1912, lviii, 274.

[3] Tscheboksaroff: Archiv für die gesammte Physiologie, 1910, cxxxvii, 59.

[4] Elliott: Journal of Physiology, 1912, xliv, 374.

[5] von Anrep: Journal of Physiology, 1912–1913, xlv, 307.

[6] Elliott: Loc. cit., p. 406.

[7] Cannon and Hoskins: American Journal of Physiology, 1911, xxix, 274. Starkenstein: Zeitschrift für experimentelle Pathologie and Therapie, 1911, x, 95. Czubalski: Zentralblatt für Physiologie, 1913, xxvii, 580.

[8] Cannon and Hoskins: American Journal of Physiology, 1911, xxix, 274. Elliott: Loc. cit., p. 406.

[9] Elliott: Loc. cit., p. 385.

[10] Cannon and de la Paz: American Journal of Physiology, 1911, xxviii, 64.

ism of secretion may be centrally excited by ether,[11] chloroform,[11] morphine,[11] strophanthin[12] and digitoxin[12] and peripherally by nicotine[13] and pilocarpine.[14]

It is noteworthy that the experiments which form the basis for the above statements yield no evidence of the existence of a nervous mechanism for inhibiting suprarenal secretion other than that which is implied in cessation or absence of stimulation, exhaustion of the glands, or alterations in blood flow through the glands. Having in mind the close relationship between the centers for suprarenal secretion and the vasoconstrictor mechanism as emphasized by Elliott and von Anrep, the thought occurred to us that inhibition of suprarenal secretion might be produced by influences known to inhibit the vasoconstrictor center. The most clean cut and constant example of inhibition of the vasoconstrictor center is to be found in its reaction to stimulation of the depressor nerve. The experiments described in this paper were therefore designed to answer the question, "Does stimulation of the depressor nerve result in a decrease in the rate of discharge of epinephrine from the suprarenal gland: if so, can it be attributed to an influence exerted upon the secreting structures rather than to effects upon blood flow through the gland?" We believe that evidence has been secured which permits an affirmative answer to both parts of the question.

TECHNIQUE

The experiments were performed on rabbits, prepared for collection of blood from the suprarenal veins according to Dale's description of Biedl's method.[15] They were narcotized by introduction of 1.8–2.0 grams of ethyl urethane per kilo into the stomach: a little ether was given by inhalation during the operation: no ether was necessary during the experimental period. The carotid artery, left external jugular vein, vagus and depressor nerves were exposed. A cannula was inserted into the carotid artery for recording blood pressure, and

[11] Elliott: Loc. cit., p. 383.

[12] Richards and Wood: Journal of Pharmacology and Experimental Therapeutics, 1915, vi, 283.

[13] Cannon, Aub and Binger: Journal of Pharmacology and Experimental Therapeutics, 1912, iii, 379.

[14] Dale and Laidlaw: Journal of Physiology, 1912–1913, xlv, 1.

[15] Dale and Laidlaw: Journal of Physiology, 1912–1913, xlv, 1. Biedl: Archiv für die gesammte Physiologie, 1897, xlvii, 481.

into the peripheral stump of left external jugular vein. Both vagus nerves were cut. The depressors were identified by the results of electrical stimulation. In some experiments the left median nerve was prepared for central stimulation.

The abdomen was opened by a long median incision, the coeliac axis, superior and inferior mesenteric arteries ligated and the animal eviscerated. The renal arteries and veins and the spermatic (or ovarian) veins were tied. The abdominal aorta was ligated just below the renal arteries. After ligating the inferior vena cava a cannula was inserted into its central stump just below the entrance of the renal veins. A loose ligature was placed about the inferior vena cava above the entrance of the right suprarenal vein. Fifty mgm. of hirudin per kilo, dissolved in salt solution, were injected into the vena cava after the operation was completed and before any samples of blood were taken. The temperature of the animal was maintained throughout the experiment by an electric heating pad. In some experiments artificial respiration was maintained by a Meyer pump: in others, natural respiration was adequate.

For the purpose of estimating epinephrine output of the glands blood was drawn from the suprarenal veins by tightening the ligature about the inferior cava above the suprarenal veins and simultaneously opening the clamp on the cava below the suprarenal veins. In experiments 1, 2, 3 and 5 the blood was collected in a pipette temporarily attached to the cannula in the cava by a short piece of rubber tubing; in the others, the cannula in the cava was made from a 2 cc. graduated pipette; the neck of the cannula was made as close as possible to the zero mark and a side tube, by which the pipette could be emptied, was sealed in at that point. In experiments 5–11 we endeavored to make the duration of collection of blood samples from the suprarenal veins the same for each sample by placing a finger over the open end of the pipette and retarding to a greater or less extent the flow of blood into it. The purpose was to make the rate of flow of blood through the glands constant. It seems hardly possible that this purpose was adequately accomplished on account of the distensibility of the walls of the veins in the space bounded by the gland capillaries, loose ligature and cannula. In the other experiments, therefore, the blood was allowed to flow freely, the duration of the period of collection being recorded on the kymograph by an electric signal and the volume of blood accurately measured.

Stimulation of the depressor was produced by an interrupted current of moderate strength which could be borne by the tongue without discomfort. In collecting blood during depressor stimulation, the stimulus was applied 15–30 seconds before collection begun and was maintained until it was finished.

In experiments 1–16 the lumbar divisions of the suprarenal veins were not ligated. In experiment 17, this was done on the left side; in experiment 18, the lumbar divisions of both suprarenal veins were completely ligated close to the gland and we therefore believe that in this experiment blood was secured from the suprarenal veins exclusively. The agreement of the results of experiments 18 and 17 with those of the earlier experiments leads to the conclusion that mixture of suprarenal blood with that from other sources has not been a factor of importance in the results obtained.

The epinephrine content of various samples of blood was tested by means of the isolated intestinal muscle method as employed by Cannon and de la Paz.[16] The muscle used was excised from the intestine of a deeply chloroformed cat,[17] prepared in a dish of oxygenated Ringer's solution at 37°C. and mounted in Ringer's solution in a muscle chamber of about 3 cc. capacity so that its contractions could be recorded. A slow current of oxygen continually bubbled through the fluid in the chamber. The chamber was immersed in a large water bath, in which were suspended also the tubes containing blood samples to be tested. Changes in muscular contraction due to temperature variations were, we believe, excluded.

Each sample of blood was oxygenated for two or three minutes before being tested.

As a routine, the intestinal strip was allowed to remain in Ringer's solution until its contractions became reasonably uniform. Then control blood—that drawn from peripheral stump of the external jugular or, in experiments 1 and 2, from the inferior cava—was substituted. When the muscle contractions in this medium had become uniform, the samples from the suprarenal veins were tested in the order in which they were drawn. Between each two tests of suprarenal blood, the muscle was immersed in control blood. In the later experiments of the series all blood samples were accurately diluted with Ringer's solution, made up without bicarbonate.

[16] Cannon and de la Paz: American Journal of Physiology, 1911, xxviii, 64.

[17] In our experience the cat's intestinal muscle has proved to yield more active, stable and uniform preparation than that of the rabbit.

RESULTS

The abbreviated protocols of nine experiments are given at the end of the paper. They agree in showing that the intensity of the reaction for epinephrine in blood collected from the suprarenal veins is lessened if collection of the blood is made during stimulation of the depressor nerve. The degree of this effect is variable. In experiment 2 the blood collected during depressor stimulation caused no inhibition of the intestinal strip, whereas blood collected five minutes later under identical conditions save that the depressor nerve was not stimulated caused complete inhibition of both tonus and rhythmic contractions. In other experiments the difference is apparently slight; the suprarenal blood taken when the depressor nerve was not stimulated gave complete inhibition of tonus and contractions while that similarly collected during stimulation of the depressor gave inhibition of tonus with only partial inhibition of contractions. On cursory examination of the record, such a difference as this between two samples of blood might appear to be too slight to be worthy of serious consideration. It is to be remembered, however, that the maximal reaction of the intestinal muscle to epinephrine is complete inhibition: obviously the method, without modification, gives no means of recognizing whether a sample of blood contains more than the minimal amount of epinephrine sufficient to give the maximal reaction. Evidence in this connection is found in the record of experiments 14, 17 and 18. In experiment 14 (fig. 7) blood sample 5 taken during depressor stimulation is compared with samples 4 and 6 taken in the absence of depressor stimulation. When first tested, 4 and 6 gave complete inhibition, 5 showed inhibition of tonus which was interrupted by rhythmic contractions. All three samples were allowed to stand for about twenty minutes under identical conditions. When tested the second time, 4 and 6 yielded a reaction which was just short of maximal, while sample 5 yielded a result which could hardly be called a positive reaction. The spontaneous disappearance of epinephrine from the three samples was just sufficient to reveal the marked excess of that substance in samples 4 and 6 over sample 5. Similarly in experiment 17 (fig. 8) the first comparison of samples 4 and 5 showed no difference in intensity of reaction. Further dilution and standing (4a and 5a) showed that sample 5 which was collected during stimulation of the depressor, contained decidedly less epinephrine than sample 4. Again in experiment 18 (fig. 9) successive dilution showed the great disparity in epinephrine content of blood samples 5 and 6.

It may be well to point out that the order in which blood samples were taken was not uniform and hence the observed effect of depressor stimulation cannot be explained as the result of a constant source of error such as loss of blood involved in collection of samples. In experiments 1, 11, 13 and 14 the collection of blood during depressor stimulation was both preceded and followed by the collection of a sample during no nerve stimulation. In the first part of experiment 3, the order of collection was reversed in the later as compared with the earlier part of the experiment.

In two experiments (13 and 14) an attempt was made to increase the normal output of epinephrine by stimulation of a sensory (median) nerve, with the idea that by such a procedure the decrease caused by depressor stimulation might be more striking. The results appear, however, to be of the same order as those in which this trial was not made.

In one experiment evidence of a somewhat different sort is to be found in support of the conclusion that the epinephrine content of the suprarenal blood can be lessened by central stimulation of the depressor nerve. It is known that section of the depressor nerve is not commonly followed by rise in arterial pressure. Bayliss[18] showed, however, that if plethora were induced by infusion of salt solution section of the depressors caused marked rise of pressure. These facts support the view that while the depressor is not normally in a state of excitation it may be physiologically stimulated by plethora in such a way as to take part reflexly in the adjustment of the circulation to increased volume of fluid in the blood vessels. In experiment 17, immediately after the intravenous infusion of 25 cc. of salt solution, blood sample 1 was taken from a suprarenal vein. Both depressor nerves were then cut and blood sample 2 was similarly taken as soon as possible. The reaction for epinephrine in sample 1 was negative; in sample 2, strongly positive. In this experiment section of the depressors caused a rise of arterial blood pressure of approximately 20 mm. of mercury.

In the experimental protocols at the end of this paper are included the rates of flow of blood from the suprarenal veins. On these figures is based our conclusion that the decrease in epinephrine content attendant upon stimulation of the depressor nerve is due to an effect upon secretory processes in the gland and is not the result merely of faster flow of blood through the gland. For convenience the figures, expressed

[18] Bayliss: Journal of Physiology, 1893, xiv, 303.

as fractions of a cubic centimeter per second, are tabulated below. The figures in parentheses are the numbers of the blood samples.

Rates of blood flow from suprarenal veins

EXPERIMENT NO.	1	2	3		5	
Before depressor stim.	(B_2) 0.272		(2) 0.136			
During depressor stim.	(B_4) 0.091	(2) 0.091	(3) 0.111	(7) 0.077	(2) 0.091	(5) 0.115
After depressor stim....	(B_5) 0.166	(3) 0.071		(8) 0.059	(3) 0.076	(6) 0.103

EXPERIMENT NO.	11	13		14	17	18
Before depressor stim...	(2) 0.06	(3) 0.082	(5) 0.055	(4) 0.05	(4) 0.08	(5) 0.05
During depressor stim.	(3) 0.058	(4) 0.09	(6) 0.059	(5) 0.066	(5) 0.07	(6) 0.057
After depressor stim....	(4) 0.029		(7) 0.045	(6) 0.06		

Of these figures we are inclined to attach the most value to those of experiments 17 and 18 for in these we are certain that there was the least degree of admixture of suprarenal blood with that from lumbar muscles. In experiment 17, the rate of flow was actually less during depressor stimulation than after: in experiment 18 the difference is far too small to account for the observed difference in epinephrine content.

We are reporting only nine of a series of eighteen experiments upon the subject under discussion: nine experiments have been discarded in the preparation of this report. Of these, five failed to show less epinephrine in the suprarenal blood taken during depressor stimulation than before or after. In two of these five the left splanchnic nerve was found to have been cut in the evisceration. In the four which contained positive results, the rate of blood flow from the suprarenal veins during depressor stimulation was increased to such an extent as to make it impossible to say that vascular change was not responsible for the diminution in epinephrine content. In not a single experiment has there been any evidence that the suprarenal blood taken during depressor stimulation contained more epinephrine than that taken before or after depressor stimulation.

From these results we are convinced that the processes in the suprarenal gland which are responsible for the discharge of epinephrine into the blood are subject to reflex inhibition by way of the depressor nerves: in a word, the mechanism of suprarenal secretion is involved not only in pressor but in depressor reflexes.

PROTOCOLS

In the following protocols the data for each blood collection are given in the following order: Number (designation) of blood sample; time of collection; volume of sample, its source, time which elapsed during collection, and rate of flow per second; blood pressure in millimeters of mercury at the beginning of collection.

Each tracing is to be read from left to right; contraction of the muscle is shown by the down stroke, relaxation by the upstroke of the lever. Each division of the time record represents 30 seconds and the actual time is printed at frequent intervals at the bottom of the tracing. Each tracing has been reduced to one-fourth of the size of the original. The numbers along the curves of muscle contraction are the numbers of blood samples tested: the exact time when each sample was substituted in the muscle chamber is shown by the point at which its number appears on the curve. Since the reproduction of the records has made some of the numbers almost illegible, those which designate suprarenal blood have been reprinted at the margin of the figure.

Experiment 1. October 13, 1914. Female rabbit, 2750 grams. Operations finished at 3.50 p.m. Artificial respiration. Blood samples as follows:

B_1. 4.01. 2 cc. from cava, 22 seconds, 0.091 cc. per second. B. P., 80.

B_2. 4.07. 3 cc. from suprarenal veins, 11 seconds, 0.272 cc. per second. B. P., 65.

B_3. 4.12.30. 3 cc. from cava, 18 seconds, 0.166 cc. per second. B. P., 40.

B_4. 4.21.33. 3 cc. from suprarenal veins during stimulation of left depressor (B. P., 40→25), 33 seconds, 0.091 cc. per second. B. P., 25.

B_5. 4.27.30. 3 cc. from suprarenal veins, 18 seconds, 0.166 cc. per second. B. P., 30.

B_6. 4.36.45. 3 cc. from suprarenal veins during stimulation of left depressor (B. P., 25→10), 23 seconds, 0.130 cc. per second.

In this experiment beginning with B_2, 3 cc. of Ringer's solution were injected into the jugular vein after each collection of blood.

The tracing from this experiment is shown in figure 1, page 62.

Experiment 2. October 15, 1914. Male rabbit, 2425 grams. Operation finished at 4.01 p.m. Artificial respiration. Blood samples taken as follows:

1. 4.15. 3 cc. from inferior cava; 43 seconds, 0.07 cc. per second. B. P., 40.

2. 4.27.52. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 50→30), 33 seconds, 0.091 cc. per second. B. P., 30.

3. 4.32.40. 3 cc. from suprarenal veins, 42 seconds, 0.071 cc. per second. B. P., 35.

Between the collection of samples 2 and 3, 3 cc. of Ringer's solution were injected into the external jugular vein.

The tracing from this experiment is shown in figure 2, page 62.

Fig. 1. *Experiment 1.* Cat's intestinal muscle contracting in Ringer's solution. At B_1, blood sample B_1 was substituted. The effects of samples B_2 and B_5 (suprarenal blood) are to be compared with that of B_4 (suprarenal blood taken during depressor stimulation).

Fig. 2. *Experiment 2.* Cat's intestinal muscle contracting in Ringer's solution. Substitution of various blood samples is indicated by the figures below the tracing.

Experiment 3. October 16, 1914. Female rabbit, 2815 grams. Operations finished at 1.33 p.m. Artificial respiration. Blood samples taken as follows.

1. 1.26. 2 cc. from external jugular, 120 seconds, 0.016 cc. per second. B. P., 75.
2. 1.36.20. 3 cc. from suprarenal veins, 22 seconds, 0.136 cc. per second. B. P., 75.
3. 1.51.10. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P. 60→40), 27 seconds, 0.111 cc. per second. B. P., 40.
4. 2.10.15. 3 cc. from suprarenal veins, 39 seconds, 0.077 cc. per second. B. P., 45.
5. 2.17.15. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 42→18), 31 seconds, 0.097 cc. per second. B. P., 18.
6. 2.43. 3 cc. from inferior cava, 29 seconds, 0.104 cc. per second. B. P., 60.
7. 2.46.25. 2 cc. from suprarenal veins during a stimulation of left depressor nerve (B. P., 55→30), 26 seconds, 0.077 cc. per second. B. P., 30.
8. 2.52.35. 2 cc. from suprarenal veins, 34 seconds, 0.059 cc. per second. B. P., 40.
9. 3.00. 3 cc. from external jugular vein.

Between blood collections 5 and 6, 20 cc. of Ringer's solution were injected into the inferior vena cava.

The tracing from this experiment is shown in figure 3, page 63.

Fig. 3. *Experiment 3.* Cat's intestinal muscle contracting in blood sample 1. Substitutions of blood samples were made as indicated by figures under the tracings. The designation *RR* under the test of blood sample 2 means that the muscle was washed with Ringer's solution at the points indicated. At the beginning of the second portion of the tracing the muscle was contracting in blood sample 9.

Experiment 5. October 19, 1914. Female rabbit, 2825 grams. Operations finished at 3.12 p.m. Artificial respiration. Blood samples taken as follows:

1. 3.18. 3 cc. from external jugular, 80 seconds, 0.037 cc. per second. B. P., 48.

2. 3.27.30. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 40→25), 33 seconds, 0.091 cc. per second. B. P., 25.

3. 3.42.15. 2.5 cc. from suprarenal veins, 33 seconds, 0.076 cc. per second. B. P., 35.

4. 4.23. 3 cc. from external jugular. B. P., 28.

5. 4.37. 3 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 40→18), 26 seconds, 0.115 cc. per second. B. P., 18.

6. 4.40.30. 3 cc. from suprarenal veins, 29 seconds, 0.103 cc. per second. B. P., 30.

7. 4.43. 2.5 cc. from external jugular. B. P., 25.

Between the collections of samples 3 and 4, 20 cc. of Ringer's solution were injected into the inferior vena cava.

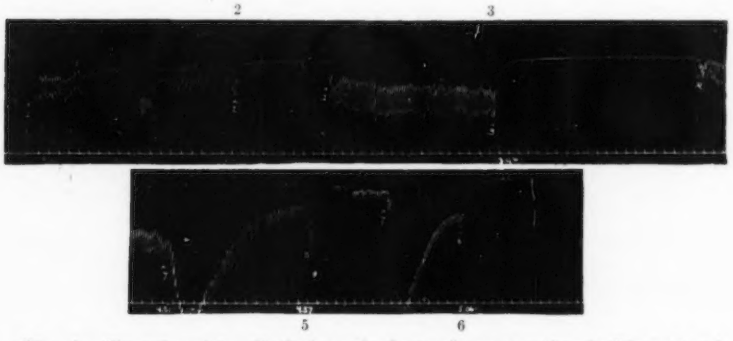

Fig. 4. *Experiment 5.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures under the tracing. At the beginning of second portion of the tracing the muscle was contracting in blood sample 2 diluted with equal volume of Ringer's solution.

Experiment 11. November 19, 1914. Male rabbit, 1800 grams. Operations finished at 3.50 p.m. Natural respiration. Blood samples taken as follows:

1. 4.14. 3.2 cc. from external jugular, 7 minutes. B. P., 80.
2. 4.29. 1.8 cc. from suprarenal veins, 30 seconds, 0.06 cc. per second. B. P., 60.
3. 4.37. 1.8 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 40→28), 31 seconds, 0.058 cc. B. P., 28.
4. 4.50. 1.5 cc. from suprarenal veins, 51 seconds, 0.029 cc. per second. B. P., 22.

Fig. 5. *Experiment 11.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures under the tracing.

Experiment 13. November 21, 1914. Male rabbit, 2100 grams. Operations finished at 11.43 a.m. Natural respiration. Blood samples taken as follows:

1. 11.57. 4.4 cc. from external jugular. B. P., 98.
2. 12.01. 3.4 cc. from suprarenal veins, 20 seconds, 0.170 cc. per second. B. P., 85.
3. 12.08.30. 1.8 cc. from suprarenal veins during central stimulation of left median nerve (B. P., 88→80), 22 seconds, 0.082 cc. per second. B. P., 80.
4. 12.13.30. 1.8 cc. from suprarenal veins during simultaneous stimulation of left median and of both depressor nerves (B. P., 70→65→47), 20 seconds, 0.09 cc. per second. B. P., 47.
5. 12.36. 2.2 cc. from suprarenal veins, 40 seconds, 0.055 cc. per second. B. P., 48.
6. 12.46. 1.6 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 55→45), 27 seconds, 0.059 cc. per second. B. P., 45.
7. 12.52. 1.8 cc. from suprarenal veins, 40 seconds, 0.045 cc. per second B. P., 30.

Fig. 6. *Experiment 13.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures under the tracing. All blood samples were diluted with equal volume of Ringer's solution.

Experiment 14. November 24, 1914. Female rabbit, 2050 grams. Operations finished at 11.22 a.m. Natural respiration. Blood samples taken as follows:

1. 11.39. 4.6 cc. from external jugular. B. P., 85.

2. 11.52. 2.9 cc. from suprarenal veins, 26 seconds, 0.111 cc. per second. B. P., 80.

3. 12.01. 2.1 cc. from suprarenal veins during stimulation of left depressor nerve (B. P., 83→60), 25 seconds, 0.084 cc. per second. B. P., 60.

4. 12.11. 1.5 cc. from suprarenal veins during central stimulation of left median nerve (B. P., 75→70), 31 seconds, 0.05 cc. per second. B. P., 70.

5. 12.13. 1.8 cc. from suprarenal veins during simultaneous stimulation of left median and left depressor nerves (B. P., 80→70→50); 27 seconds, 0.066 cc. per second. B. P., 50.

6. 12.15. 1.8 cc. from suprarenal veins during central stimulation of left median nerve (B. P., 80→57→80), 30 seconds, 0.06 cc. per second. B. P., 80.

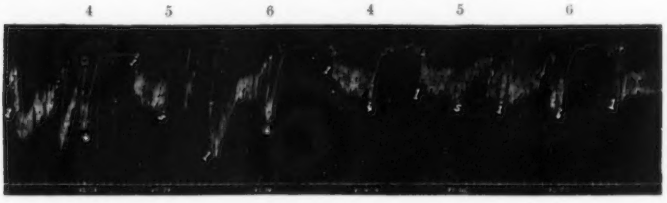

Fig. 7. *Experiment 14.* Cat's intestinal muscle contracting in blood sample 3. Other blood samples substituted as indicated by figures under the tracing. All blood samples were diluted with equal volume of Ringer's solution. During the interval between the first and second testing of samples 4, 5 and 6, they were kept in the air at room temperature under identical conditions.

Experiment 17. January 22, 1915. Female rabbit, 2200 grams. Operations finished at 11.50 a.m. Natural respiration. Blood samples taken as follows:

1. 12.07. 2.2 cc. from suprarenal veins immediately after the infusion of 25 cc. 0.9 per cent NaCl into vena cava (12.04–12.05.40), 6¼ seconds, 0.35 cc. per second. B. P., 102.

2. 12.11. 2.1 cc. from suprarenal veins immediately after section of depressor nerves (B. P., 100→120), 9⅓ seconds, 0.225 cc. per second. B. P., 100.

3. 12.13. 3.5 cc. from external jugular vein. B. P., 110.

4. 12.38. 2 cc. from suprarenal veins, 25 seconds, 0.08 cc. per second. B. P., 70.

5. 12.44. 2.1 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 90→62), 30 seconds, 0.07 cc. per second. B. P., 62.

The tracing from this experiment is shown in figure 8, page 66.

Experiment 18. January 23, 1915. Rabbit, 1930 grams. Operations finished at 11.59 a.m. Blood samples taken as follows:

1. 12.35. 2 cc. from suprarenal veins immediately after infusion of 25 cc. of 0.9 per cent NaCl into inferior cava (12.30.30–12.32.10), 15 seconds, 0.133 cc. per second. B. P., 100.

2. 12.38. 2.2 cc. from suprarenal veins immediately after section of both depressor nerves (B. P., 100→118), 20 seconds, 0.110 cc. per second. B. P., 125.

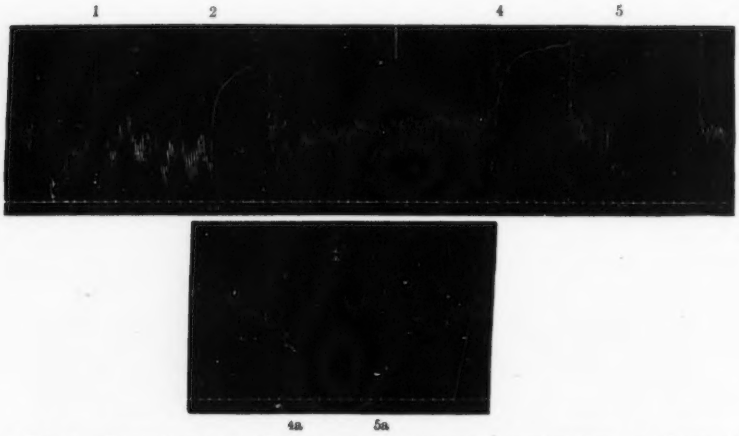

Fig. 8. *Experiment 17.* Cat's intestinal muscle contracting in Ringer's solution. Blood samples substituted as indicated by figures on tracing. In the tests shown in the first portion of tracing each blood sample was diluted with equal volume of Ringer's solution. In the second tests of bloods 4 and 5 (4a, 5a, second portion of tracing) each sample was diluted with 3 volumes of Ringer's solution. During the interval between the first and second tests of bloods 4 and 5 they were kept at room temperature under identical conditions.

3. 12.42. 1.8 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 120→120), 37½ seconds, 0.048 cc. per second. B. P., 120.

4. 12.43.30. 5.5 cc. from external jugular vein. B. P., 105.

5. 1.16. 2.2 cc. from suprarenal veins, 44 seconds, 0.05 cc. per second. B. P., 90.

6. 1.19. 2.3 cc. from suprarenal veins during stimulation of both depressor nerves (B. P., 85→70), 40 seconds, 0.057 cc. per second. B. P., 70.

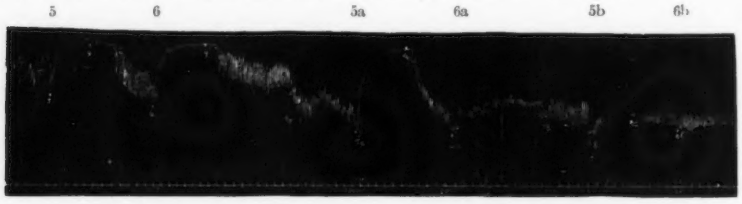

Fig. 9. *Experiment 18.* Cat's intestinal muscle contracting in blood sample 4 diluted with equal volume of Ringer's solution. Figures 5 and 6 indicate the substitution of samples 5 and 6, each diluted with one volume of Ringer's solution. Figures 4a, 5a, and 6a show tests of those samples diluted with 3 volumes of Ringer's solution. Figures 4b, 5b, and 6b indicate tests on the same samples, each diluted with 7 volumes of Ringer's solution.

A RESPIRATORY CHAMBER FOR SMALL ANIMALS

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The respiratory chambers which we wish to describe were specially devised for several lines of work which we desired to undertake in this laboratory. The central idea in all of this work depended on the development of an apparatus in which it would be possible to keep animals in a given atmosphere of oxygen and nitrogen continuously for a week or more. The chambers are suitable for small dogs, rabbits, cats, rats, mice, etc. It was necessary to have an apparatus which should be entirely automatic in regard to the oxygen supply and which would not require constant watching. It was not necessary for the work which we had in mind to determine the energy exchange. Although the apparatus is of particular interest in connection with our own work it seemed to be of sufficient general interest to warrant separate publication. We have built three chambers, two of galvanized iron and one of 3.18 mm. boiler plate steel. The larger one built of lighter material will be described first.

The larger chamber. This chamber is 90 cm. x 90 cm. x 45 cm. and is built of No. 22 B and S. gauge (0.635 mm.) (figs. 1 and 2), galvanized iron. There are two windows on opposite sides of the box, 45 cm. x 30 cm. of double thickness glass held by strips of metal bolted through the sides of the box and made air tight by means of a 3 mm. rubber gasket, cement, and finally a coating of wax. There is an opening at the top of the box, 30 cm. x 30 cm. centrally placed. This opening is fitted with a door which is completely removable. The door is of heavy glass set in a brass frame and is held air-tight by means of six clamps which are easily opened so that the door can be taken off within a few seconds. A heavy rubber gasket is placed between the brass frame of this door and the brass casting which receives it. There is no trouble in getting this door air tight. The box is well lighted with the two large glass windows and glass door above. There is a small opening in the bottom through which urine or any fluid can be readily drained

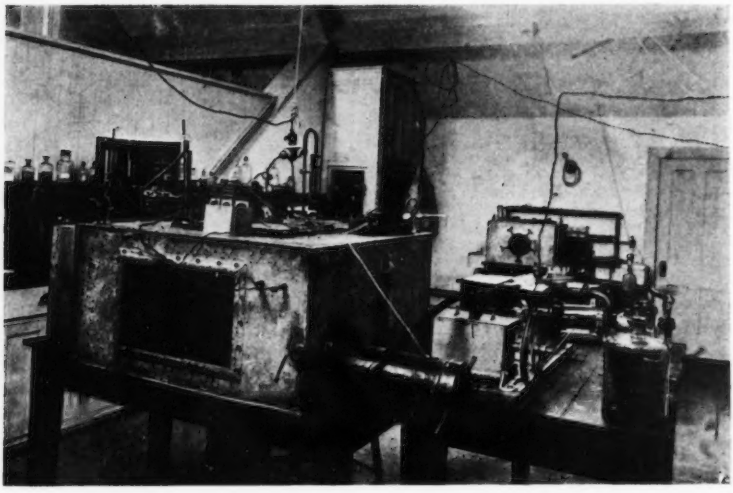

Fig. 1

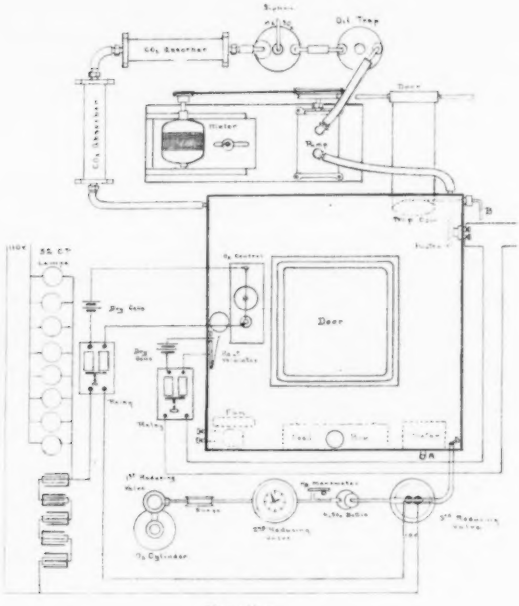

Fig. 2

off, and if it were desirable obvious arrangements for flushing the box, from time to time, without opening it could be easily arranged. The arrangements for feeding depend on the animal used. There is a water sealed pipe leading through the side of the box to supply water (*A*, fig. 2). When rabbits are used we place in the box a hopper filled with corn, oats and ground alfalfa which feeds from the upper compartment into the lower. Enough feed may be placed in the hopper to last four rabbits for four days. To refill the hopper an opening was made in the top of the box, into which a supply pipe into the upper part of the hopper fits. This opening is closed by means of a large rubber stopper (5 cm. in diameter) and the food can readily be supplied for another four days by removing the stopper. This requires only a few seconds and the influence on the composition of the atmosphere of the box is entirely negligible.

Extending out from the box on the side on which the absorbing apparatus is placed is a trap made of iron piping 38 cm. long and 18 cm. in diameter. It is closed toward the outside by means of a door which screws on, a heavy rubber gasket being used between the bearing surfaces. This door is of glass with a heavy iron frame. At the entrance of the trap from the chamber is placed a door which may be opened and closed by means of a lever (*B*, fig. 2). The lever passes through a stuffing box to the outside. The trap serves a double purpose. When rabbits are used in the box they may be removed and examined without altering the composition of the atmosphere of the chamber by more than 1 per cent. This is done by closing the trap toward the interior of the chamber and removing the door. A carrot is placed just inside the trap and the door replaced. Then the trap is opened toward the interior of the chamber. Within a few minutes the rabbit goes into the trap attracted by the carrot, when the damper is closed and the rabbit is removed for observation. In case the rabbits are too timid to venture into the trap they can always be removed by a snare operated through the trap. Small dogs can also be readily removed through the trap. They come out willingly when given the opportunity. The other purpose of the trap is for the introduction of any special food material or drug from time to time. The apparatus for the absorption of the water and carbon dioxide produced by the animals is taken from Benedict[1] so that no description is required. We have, however, introduced three features of great convenience into the absorbing system, as follows:

[1] Benedict: Deutsch. Arch. f. klin. Med., 1912, cvii, 156.

First. Traps for the sulphuric acid bottle. (a) The tube entering the sulphuric acid bottle has a large bulb of 500 cc. capacity. In case the rubber tubing between the pump and sulphuric acid bottle should break this bulb would receive the sulphuric acid forced up by the back pressure in the rest of the absorbing system and prevent the concentrated acid from reaching the current of air from the pump and being sprayed over the room. (b) The outlet tube from the sulphuric acid bottle is provided with a trap having a large inner tube. The tube is lipped

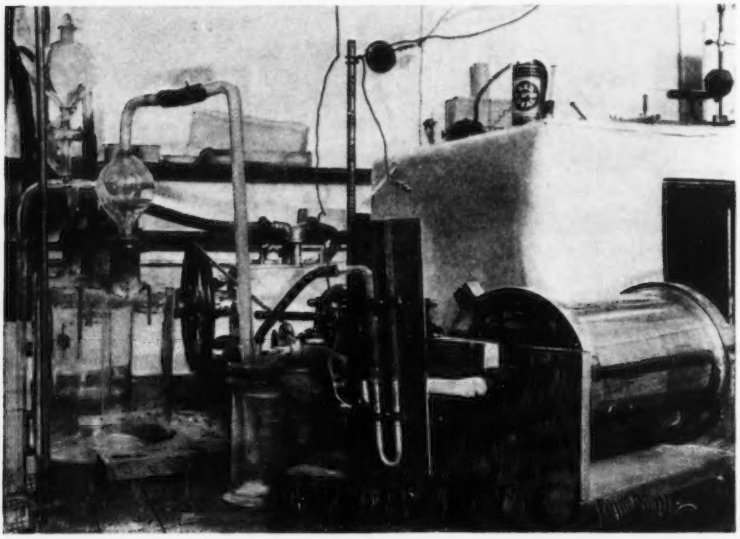

Fig. 3

at the bottom and allows the acid reaching the tube to flow back into the bottle without obstructing the current of air. This trap effectively prevents any acid from being carried over.

Second. In experiments of long duration it is a great convenience to be able to remove the spent sulphuric acid and to replace it with fresh acid without removing the stoppers. For this purpose the sulphuric acid bottle is provided with a combined siphoning tube and separatory funnel as shown in figure 3.

Third. A small bottle or T-tube is inserted between the sulphuric acid bottle and the soda-lime container (fig. 3). This bottle is con-

nected with a mercury manometer having a platinum wire fused into the glass and in contact with the mecury. Another platinum wire passes down the open end of the manometer. During the time that the apparatus is operating there is a positive pressure in the bottle—usually of about 10 mm. mercury. In case the soda-lime becomes caked and offers resistance to the passage of the air stream, it will be readily noted by the manometer reading. In case anything occurs to interfere with the circulation of the air, the manometer reading drops to zero and the mercury makes contact with the two platinum wires and closes a dry cell circuit which rings an electric bell. This obviates constant observation of the apparatus during the working hours. At night the wiring is slightly changed so that the first vibration of the electric bell releases a telephone connected with the university central (fig. 4). The signal from this 'phone is recognized by the university operator as a trouble signal and we are immediately notified. This obviates the necessity for having a man on duty constantly day and night during experiments.

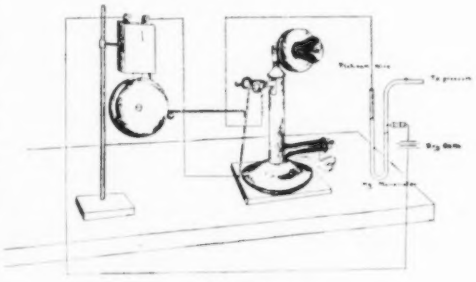

Fig. 4

The supply of oxygen. This is automatically controlled as follows: A 10 cm. tambour covered with rubber dam of proper thickness is connected with the interior of the box. A decrease of the pressure within the box draws the rubber dam down. There is a brass arm bearing a platinum point resting upon a wedge glued to the center of the rubber dam. When the dam is drawn down, this arm closes a relay circuit which in turn breaks a circuit operating on the third oxygen valve described below. This is a slight modification of the method proposed by Williams.[2] Oxygen cylinders containing oxygen under a pressure of 100 to 150 atmospheres supply the oxygen as needed. The pressure is lowered by means of three reducing values. The first valve which is supplied with the cylinder reduces the pressure to approximately 6 atmospheres or less. We have inserted a gauge just beyond the

[2] Williams: Journ. Biol. Chem., 1912, xii, 317.

valve so that the exact pressure at this point is known. The second and third reducing valves (figs. 5 and 6) were built by our mechanician from Mason reducing valves on the market. The first valve will operate between pressures of from 15 atmospheres to less than 1 atmosphere. We, however, never allow less than one atmosphere of pressure on the cylinder side of this valve. This valve is provided with a dial and pointer so that it may be set to deliver the oxygen towards the box at any desired pressure. In most of our work it was set to deliver oxygen at a pressure of approximately 30 mm. of mercury. A mercury manometer is placed between the second and third reducing valves in order to control perfectly the setting and working of this valve. The third valve opens or closes with changes of pressure within the box of 0.2 mm. of water. The electric control of this valve has already been described. When the pressure in the box falls due to the utilization of oxygen and the absorption of the water and carbon dioxide produced by the animal, contact is made between the tip of the tambour lever and the mercury cup. This actuates the relay which breaks the battery current. The valve then opens admitting oxygen into the box until the tip of the tambour lever leaves the mercury cup, when it is again closed. The box can be set for a pressure slightly above or slightly below atmospheric pressure by changing the position of the tambour lever on the upright, or by raising or lowering the mercury cup. The box is provided with: A wet and a dry bulb thermometer and a simple arrangement for moistening the wet bulb; a 20 cm. electric fan playing on the thermometers in order to insure the maximum lowering of the wet bulb thermometer and to insure a uniform atmosphere in the box; an electric heater of nichrome wire controlled by a delicate heat regulating mechanism. This heater is not constructed to adjust for a very great fall in the temperature of the room and it is essential that the temperature of the room in which the chamber is placed should not vary more than 5°C. during an experiment. The temperature of the box is usually

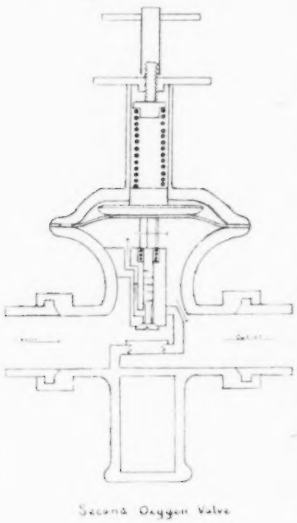

Fig. 5

about 1°C. above that of the room during experiments. The importance of preserving a uniform temperature in the box will readily be understood when it is remembered that the system is entirely closed and the inflow of oxygen depends on changes in pressure within the system. Thus, if the temperature within the chamber falls, the per cent of oxygen will rise, whereas if the temperature rises the oxygen content will fall.

In the first work which we desired to do, the animal was to be placed in an atmosphere poor in oxygen at the ordinary atmospheric pressure and with as nearly as possible the normal humidity and carbon dioxide content of the outside air. The problem then presented itself as to the best method for quickly reducing the oxygen and raising the nitrogen content of the atmosphere of the box. We tried burning alcohol, phosphorus and illuminating gas, but all were unsatisfactory because of the production of pharmacologically active products which could not be completely absorbed. In the combustion of ethyl alcohol we found that aldehyde is produced among the products of oxidation as the oxygen concentration falls. Furthermore, alcohol and illuminating gas burn less and less vigorously as the oxygen content of the atmosphere decreases and they cease to burn at about 16 per cent and 13 per cent oxygen respectively.[3] We also tried passing the air over highly heated copper turnings but found the method unsatisfactory. We found that by burning hydrogen in the atmosphere of the chambers we could reduce the oxygen content rapidly and in a manner entirely free of objections. During the time that the hydrogen is burned the chamber is left in communication with the outside air through a tube inserted at *M* (fig. 1), so that as the oxygen is absorbed by the flame and the water produced was removed by condensation and concentrated sulphuric acid, atmospheric air was constantly flowing into the chamber. We constructed a very convenient piece of apparatus for the burning

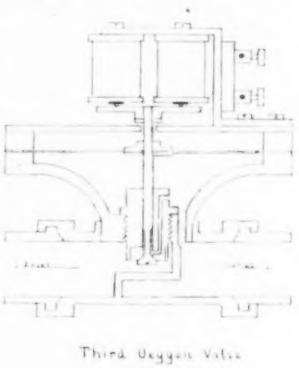

Fig. 6

[3] Illuminating gas varies so much in composition in different localities that it is not possible to state the oxygen percentage at which the flame is extinguished. The Madison illuminating gas is extinguished at about 13 per cent oxygen.

of the hydrogen and the condensation of the water (fig. 7) which is inserted in the absorbing system between the carbon dioxide absorber and the respiratory chamber. The chamber *A* in which the hydrogen is burned is 40 cm. high and 25 cm. in diameter. It is provided with a window, 4 cm. in diameter through which the flame could be observed. The volume of air delivered by the pump into the chamber varies from 32 to 52 liters a minute, depending on the speed of the pump. The current of air passes through the tube *B* and is directed toward the floor of the chamber. The air escapes from the chamber at the top and passes through the coil *C* and out at *D*. The sides of the chamber are continued upward 15 cm. so that the coil may be completely immersed in water and ice. The air then passes through the condensing chamber *E* which is packed in ice. It is shown in the dia-

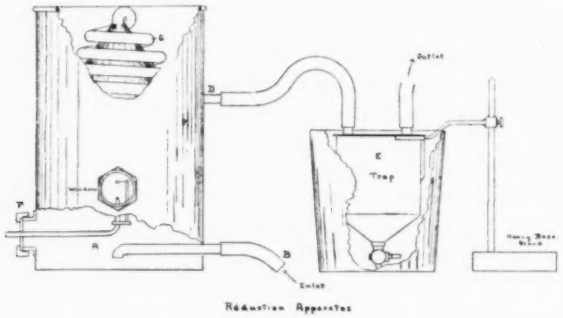

Fig. 7

gram in place in the pail. This apparatus is inserted without disconnecting any rubber tube. The hydrogen is burned from a brass tip and the flame impinges on a loop of platinum in order to make it luminous. This tip is held in place by a union, *F*. The hydrogen may be obtained from a Kipp apparatus or from a cylinder of hydrogen. Electrolytic hydrogen is now readily obtainable in most places. The advantages of hydrogen for reducing the oxygen are as follows: (1) The only product of the combustion is water. (2) If the flame is accidently extinguished for a short interval the hydrogen does not interfere with the experiment since it is physiologically inert. (3) The hydrogen continues to burn until the oxygen in the atmosphere is reduced to about 6.6 per cent.

Using this apparatus we can reduce the oxygen in the large box to

10 per cent in thirty-five minutes and in the smaller box in twenty minutes.

A second method of reducing the oxygen in the chamber which we have used very extensively is to allow the animals to reduce the oxygen themselves. The rate of the reduction depends upon the rate of oxygen utilization by the animals. This method has the advantage that it enables the animals to become accustomed more gradually to the reduced partial pressure of oxygen but it occasions unnecessary loss of time.

A third method which would be free of objection would be to sweep the air out using compressed nitrogen, but unfortunately pure nitrogen is not always readily obtainable.

Chambers for work at pressures above and below atmospheric pressure

The smaller chamber which is 50 cm. x 50 cm. x 38 cm. is similar in its general appointments to the larger one except that it is made of heavier material, No. 18 galvanized iron (1 mm.). There are two round windows on opposite sides, 14 cm. in diameter. It is provided with an electric light of 2 c.p. which is only used during observations. This box is not provided with a trap for removing the animals. Both this chamber and the one previously described are covered with 2.5 cm. of wool pipe insulation material, then with heavy canvas and painted.

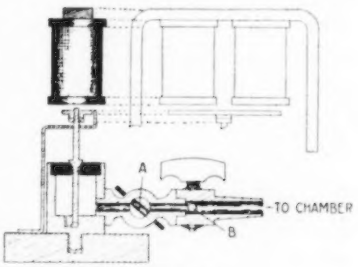

Fig. 8

The smaller chamber will stand a positive or negative pressure of about one-third of an atmosphere. The third chamber of 3.18 mm. boiler plate steel is oxy-acetyline welded along the seams. The chamber is round, the top being slightly concave and the bottom slightly convex. It is 76 cm. in diameter and 38 cm. in height. There are two round windows opposite one another and one in the center of the top, all 12 cm. in diameter. These windows are of 12 mm. plate glass, reinforced from the outside. This chamber was designed for work at reduced pressures and will stand complete evacuation. It will also stand a positive pressure of 10 kilograms per sq. cm. In the work at reduced pressure we have employed a Crowell rotary pump as in the experiments

at atmospheric pressure. Air is admitted to the chamber through the mechanism shown in figure 8. This mechanism has two openings, one controlled by a valve (*A*), and the other communicating continuously with the outside air (*B*), the size of both openings being regulated by stopcocks. Since the working principle of this mechanism depends on the gradual increase in negative pressure beyond the desired point, the stopcock controlling *B* is set so that this will occur. The other opening (*A*), which is controlled by the valve is alternately opened and closed by an electromagnet accordingly as the negative pressure increases and decreases. The current flowing through the coils of the electromagnet is automatically opened and closed by means of a relay. The relay circuit is in turn opened and closed through the contacts in the mercury manometer, which consist of platinum wire, one sealed in the side and the other introduced in the open end. The entrance of air and the pressure within the chamber is thereby automatically controlled and requires no attention whatever. The same alarm system described in connection with the first chamber is also in use with this chamber.

The results of our work with the chambers will be presented in subsequent communications.

THE MECHANISM ADAPTING THE OXYGEN CAPACITY OF THE BLOOD TO THE REQUIREMENTS OF THE TISSUES

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In 1878 Paul Bert[1] made the remarkable prediction that the blood of man and animals living at high altitudes would be found to have a greater oxygen capacity than that of corresponding individuals living at lower levels. He surmised that the cause of this increase in the oxygen carrying power of the blood would be found to be the decrease in the partial pressure of oxygen in the atmosphere respired, and that this constitutes the important feature of acclimatization to high altitudes. He believed that this adaptation would occur only after several generations of residence at high altitude. In 1882 Bert[2] confirmed his prediction and made the fundamental observation that the oxygen carrying power of the blood is much enhanced in animals living at high altitudes. The increase in the oxygen capacity of the blood could only be attributed to an increase in its haemoglobin content.

Viault[3] in 1890 showed in man that on passing from a lower to a higher altitude there occurs within a short time a marked increase in the erythrocytes in the unit volume of blood. This completed Bert's fundamental discovery. The observations of Bert and Vaiult have been controverted by a few investigators but at the present time the evidence that the increases in erythrocytes and haemoglobin do occur is overwhelming. The physiological significance and the mechanism of these blood changes have been the subject of a great number of investigations

[1] Bert: "La pression barometrique," Paris, 1878, p. 1108.

[2] Bert: Compt. rend. de l'Acad. d. Sci., 1882, xciv, 805. Bert states that Jourdanet was of the opinion that mountain sickness is due to diminished oxygen in the blood consequent to the decreased oxygen tension of the atmosphere and he designated that state of the organism as "anoxyhémie."

[3] Viault: Compt. rend. de l'Acad. d. Sci., 1890, cxi, 917; 1892, xciv, 1562. Compt. rend. de la Soc. de Biol., 1892, ix, Sér. iv, 569.

and the source of much controversy. It was with the view of throwing light on these propositions that the present study was undertaken. It was our desire to determine, by methods which would be free from objection, the nature of the blood changes at decreased pressure and if possible to bring this remarkable adaptation in line with other better known if not better understood physiological phenomena.

Historical. It is not our intention to present or critically review all of the researches in this field of work. Several excellent summaries of this literature have been published. We shall only refer to such contributions as are necessary in order to present our own results in the proper perspective.

There may be said to be unanimity among the recent workers that marked increases in erythrocytes and haemoglobin occur at high altitudes. Views differ as to the physiological significance of these changes and the mechanism by which they are brought about. Cohnheim[4] is the only well-known recent worker who has expressedly maintained that the blood changes are of but little physiological significance and he has completely reversed his decision in his last communication.[5] The views in regard to the mechanism by which the blood changes are brought about may be conveniently divided into three main classes:

I. Those theories which insist that the increase in erythrocytes and haemoglobin is real and not merely relative. Two explanations of the increase have been proposed: (a) That the increase is due to increased activity of the bone marrow resulting in an increase in the erythrocytes and haemoglobin. This was the opinion held by Bert,[6] accepted by Vaiult[7] and elaborated by Miescher[8] who held that the stimulation of the bone marrow must be attributed to the low oxygen tension in the bone marrow at high altitudes. (b) That the increase is due to a lengthening of the life of the erythrocyte as advanced by Fick[9] has never received any experimental support and since everything points the other way this view may be disregarded.

II. The concentration theories according to which the increase in erythrocytes and haemoglobin per unit volume is due to increased concentration of the blood. According to this view the increase in erythro-

[4] Cohnheim: Ergebn. d. Physiol., 1912, xii, 629.
[5] Cohnheim and Weber: Deutsch. Arch. f. klin. Med., 1913, cx, 225.
[6] Loc. cit.
[7] Loc. cit.
[8] Miescher: Korrespondenzblt. f. Schweizer Aerzte, 1893, xxiii, 809.
[9] Fick: Arch. f. d. gesammt. Physiol., 1895, lx, 589.

cytes and haemoglobin is only apparent and there is no increase in the total erythrocytes and haemoglobin in the body. This view was first advanced by Grawitz[10] who maintained that there occurs increased evaporation of water from the body at high altitudes and that this results in increased concentration of the blood. If this view were correct, it would be found:

1. That the increases in erythrocytes and haemoglobin would always run parallel.

2. That since the increases in erythrocytes and haemoglobin often amount to 20 per cent, the blood would have to lose $16\frac{2}{3}$ per cent of its water and since the blood under these conditions would take up water from the tissues, in consequence of its increased tonicity, the entire body would have to lose $16\frac{2}{3}$ per cent of its water which in a man of 70 k. would mean a loss of weight of 8.2 k.

3. When the body had lost the amount of water calculated above (8.2 k) there would necessarily be a marked increase in the specific gravity of the plasma and in the material in solution.

4. A determination of the total haemoglobin in the body would show that there is no increase in the total haemoglobin.

None of these propositions are in accord with the facts. Abderhalden[11] alone found a marked parallelism in the changes in erythrocytes and haemoglobin. Some workers have found the erythrocytes increased more than the haemoglobin, while others have found the haemoglobin to show the greater increase. Another striking argument against the view that the increases are not due to loss of water by evaporation, if any further evidence were required, is to be found in the regulation of the degree of hydraemia by the kidneys which ordinarily take care of a very large variation in the water ingestion with practically no changes in the blood count. Increased evaporation without an increase in ingestion of water would result in the secretion of very concentrated urine.

The theory of Grawitz was modified by Bunge and others to meet some of the above objections. Bunge[12] supposed that vaso-constriction occurs at high altitude resulting in plasma leaving the vessels and passing into the tissues. This met the objection that there is no increase in the solid contents of the plasma at high altitudes. However, there is no evidence of such long continued vaso-constriction

[10] Grawitz: Berl. Klin. Wochensch., 1895, xxxii, 713, 740.

[11] Abderhalden: Zeit. f. Biol., 1902, xliii, 125.

[12] Bunge: Verhandl. des XIII Kongress f. innere Med., 1895, p. 192.

and the only experimental evidence favoring this view is the parallelism between the increases in erythrocytes and haemoglobin noted by Abderhalden and which has not been confirmed by other workers. The fallacy of both of these views is most clearly brought out by determinations of the total haemoglobin in experimental animals which was done by Jaquet and Suter[13] and Abderhalden.[14] Abderhalden attached no significance to the increase in haemoglobin per kilo which he found in animals at high altitude. It should be stated that Abderhalden in his more recent communication[15] on this subject states guardedly that the increases in the blood count may not be fully accounted for on the theory of increased concentration of the blood and states that new formation of erythrocytes occurs without doubt especially when there is prolonged residence at high altitude. Douglas, Haldane, Henderson and Schneider[16] in an exhaustive study of the blood changes on Pike's Peak conclude that the increased percentage of haemoglobin was apparently due in part during the first few days to concentration of the blood but afterwards entirely to a large increase in the total amount of haemoglobin which was determined by the carbon monoxide method. Schneider and Havens,[17] also working on Pike's Peak, find that the increase in erythrocytes and haemoglobin during the first two or three days at high altitude is due in part to the throwing into the general circulation of a large mass of reserve corpuscles, in part to a loss of fluid from the blood and to increased activity of the bone marrow.

III. Under this class may be grouped every other conceivable explanation of the observed facts. Thus it has been stated that the increase in the erythrocyte count at high altitude is due to a distortion of the cover slip so that the depth of the counting chamber is increased. This view is founded on faulty theoretical reasoning and has been shown experimentally to be erroneous. Again it has been held that the increases are due to unequal distribution of erythrocytes. They were supposed to be more numerous in the blood of the capillaries and smaller vessels and less numerous in the large vessels. This view has found but insignificant support experimentally and the mass of obser-

[13] Jaquet and Suter: Correspondenzblt. f. Schweizer Aerzte, 1898, xxviii, 104.
[14] Abderhalden: Zeit. f. Biol., 1902, xliii, 125.
[15] Abderhalden: Med. Klinik, 1905, 1, 210.
[16] Douglas, Haldane, Henderson and Schneider: Phil. Trans. Royal Soc., 1913, B. cciii, 185.
[17] Schneider and Havens: This Journal, 1915, xxxvi, 380.

vations opposed to it render it untenable. Finally, it has been supposed that there exists in the body a reserve or dormant supply of erythrocytes which is drawn upon at high altitude.

Methods. Previous work carried on in this laboratory has demonstrated that within certain limits the activity of the respiratory, vaso-motor and cardio-inhibitory centers may be stimulated by reducing oxygen fixation by the cells of these centers.[18] To express ourselves more accurately, the results obtained could be satisfactorily explained only on the basis of the above hypothesis. Various considerations have led us to the view that the above conclusion holds good generally for living cells and this leads directly to the proposition that the bone marrow should be stimulated by any means which will within certain limits decrease the oxygen fixation of its cells. It seemed therefore that the views of Bert and Miescher were entirely in accord with the work on the medullary centers and we determined to put them to the test.

There are many conceivable methods of reducing oxygen fixation by the bone marrow. Substances such as hydrocyanic acid which are known to reduce oxidation in living cells might be used in which case the supply of oxygen need not be reduced. There are also many methods which might be employed for reducing the oxygen supplied to the bone marrow. Nasmith and Graham[19] and Nasmith and Harrison[20] have studied the effect of chronic carbon monoxide poisoning on guinea pigs and rabbits and found marked increases in the erythrocytes and haemoglobin. Nasmith and Graham using guinea pigs state, "the effect of chronic carbon monoxide poisoning in the blood is similar to that which occurs at high altitude." Their studies were apparently not made with a view to determine the cause of the polycythaemia of altitude and are therefore the more convincing that decreased oxygen supply to the bone marrow leads to increased production of erythrocytes and haemoglobin.

Bürker, Ederle and Kircher[21] devised a different method of decreasing the oxygen supply to the bone marrow. They lessened the respiratory surface of the lungs by means of a unilateral pneumothorax. Their

[18] Grove and Loevenhart: Journ. Pharm. and Exp. Therap., 1911, iii, 131. Loevenhart: Arch. f. d. gesammt. Physiol., 1913, cl, 379. Gasser and Loevenhart, Journ. Pharm. and Exp. Ther., 1914, v, 239.

[19] Nasmith and Graham: Journ. Physiol., 1906, xxxv, 32.

[20] Nasmith and Harrison: Journ. Exp. Med., 1910, xii, 282.

[21] Bürker, Ederle and Kircher: Zentralblt. f. Physiol., 1913, xxvii, 623.

observations were made on men, dogs, and rabbits, and they found marked increases in the erythrocytes and haemoglobin. The method is open to the objection that with the collapse of one lung a marked abnormality is introduced.

In our experiments we have reduced the oxygen supply to the bone marrow by lessening the oxygen tension of the respired air. This was accomplished by two different methods, which served to check one another. First we have exposed animals to atmospheres varying in composition from 6 per cent by volume of oxygen and 94 per cent of nitrogen to 21 per cent oxygen and 79 per cent nitrogen at atmospheric pressure and second, we have worked at the corresponding tensions of oxygen obtained by partially evacuating the chamber in which the animals were kept. The apparatus in which the work was carried out is described in the preceding article together with the automatic devices which enabled us to carry out the experiments readily. In some cases we have kept the animals in the chambers described for a month only removing them every five or seven days for a few hours in order to keep the chambers in a thoroughly hygienic condition. In most of our work the animals were kept in the chambers from two to seven days. In the experiments at atmospheric pressure with reduced oxygen tension there were slight variations in the composition of the atmosphere. They were sufficiently small, however, to be disregarded entirely. They were practically always due to changes in temperature. From two to four analyses daily for oxygen and carbon dioxide enabled us to correct changes in the atmosphere before they assumed any proportions. The carbon dioxide analyses were made with Haldane's intermediate size portable apparatus for the analysis of mine gases. The oxygen determinations were made in the usual way with a Hempel phosphorus pipette as large volumes of gas could be readily taken. The importance of the experiments at atmospheric pressure using atmospheres of low oxygen tension are sufficiently obvious. Our object was to remove any mechanical factors, such as exist at reduced atmospheric pressure and which have been frequently evoked to account for the blood changes at high altitude. Thus increased evaporation of water from the body at reduced pressure has been regarded as an important factor in the high blood counts of altitude. Kronecker[22] has assumed that at high altitude the diaphragm occupies a higher

[22] Kronecker: "Die Bergkrankheit." v. Leyden's u. Klemperer's Die deutsche Klinik am Eingange des 20 Jahrhunderts 1907, 11, 130.

position and lessens the circulation through the lungs. Jacobi stated[23] that congestion of the lungs at high altitude causes a new formation of blood to fill the remainder of the vascular system. Durig and N. Zuntz[24] found that on the peak of Teneriffa their vital capacity was decreased 6 per cent to 11 per cent. Meyer[25] and Strohl[26] using the electrocardiographic method found an hypertrophy of the right ventricle at high altitudes. Heger[27] holds a view quite similar to that of Kronecker.

All theories which account for the blood changes by the reduced atmospheric pressure apart from decreased oxygen tension could be dismissed if it could be shown that the same changes occur at atmospheric pressure provided the oxygen tension is reduced by increasing the percentage of the inert gases of the atmosphere. This has been attempted by two previous investigators. Twenty years ago Sellier[28] attempted a similar solution of the problem. He used birds and guinea pigs. The experiments were apparently all of short duration. He states that he was forced to publish unwillingly before the work was completed. His apparatus was so extremely crude judging from the very meagre description that very little importance can be attached to his experimental findings. His conclusion that the polycythaemia of high altitude is due solely to low oxygen tension and that the reduced pressure, *per se*, exercises no influence, is interesting in spite of the fact that the data were not sufficient to establish the point. Recently David[29] has made similar studies. His apparatus is more elaborate but some of the methods used by him do not seem to be all that could be desired for experiments of long duration. Thus he uses potassium hydroxide for the absorption of carbon dioxide and calcium chloride for the absorption of water, both apparently in the same container. He performed a large number of experiments on animals previously rendered anaemic. The number of experiments on normal animals which he published is not sufficient to determine the reaction of normal bone marrow to oxygen want.

[23] Jacobi: Deutsch. Med. Woch., 1907, xxxiii, 17; Arch. f. exp. Path. u. Pharm., 1914, lxxvi, 423.

[24] Durig and N. Zuntz: Biochem. Zeitsch., 1912, xxxix, 435.

[25] Meyer: Journ. Med. de Bruxelles, 1912, 17, 409, 424.

[26] Strohl: Atti. d. lab. scien., A. Mosso Torino; 1912, iii, 218.

[27] Heger: Journ. Med. de Bruxelles, 1912, No. 46.

[28] Sellier: Thése, Doc. Med. Bordeaux, 1894–5.

[29] David: Zeitsch. f. klin. Med., 1912, lxxiv, 404; Deutsch; Archiv. f. klin. Med., 1913, cix, 129.

The methods of operating our apparatus are fully described in the previous paper and also the method used for obtaining the desired atmosphere for the particular experiment in our apparatus. We have performed seventy-six experiments on sixty-one animals. The animals used were as follows: twenty-three full grown, eighteeen half-grown and six young rabbits, two young pups and twelve half-grown, white rats. By far the greater number of our experiments were performed on rabbits. The samples of blood were usually taken from the marginal ear vein in rabbits and dogs but some counts were also made in rabbits from the carotid blood, or the blood in the heart, in order to determine whether there was any difference between the blood counts in the peripheral blood and that of the larger vessels. In the case of the rats the blood was obtained from the heart. The erythrocyte counts were made with the same pipettes throughout the investigation. Thoma-Zeiss counting chambers were used exclusively and here again we have used the same chambers throughout the work. Thus, since we were concerned with changes in the blood count and not absolute values, errors due to apparatus are excluded. The diluting solution used was principally a 5 per cent solution of magnesium sulphate. In the case of very young animals it was found necessary to slightly modify this solution in order to prevent the destruction of erythrocytes. All haemoglobin determinations were made with the same v. Fleischl-Miescher haemoglobinometer, the same Miescher pipette being employed throughout. All erythrocyte counts and haemoglobin determinations were made at least by two and often by all three of us, and the average of the results was taken. In case of any marked disagreements new determinations were made. In four of the rats, two at low oxygen tension and two controls at normal oxygen tension, determinations of the total haemoglobin were made. The animals were anaesthetized with ether and were perfused with Ringer's solution through the aorta until the perfusate was no longer blood stained. The tissues were then cut up and extracted with the same solution. The haemoglobin was then determined in the total mixture using the Miescher method. Everytime blood was taken smears were prepared and studied later. Jenner stain was employed, because of its characteristic staining of newly formed erythrocytes. Many of the rabbits were killed at the end of the experiments and the bone marrow and various other tissues preserved in formaldehyde or Zenker's fluid for histological study.

EXPERIMENTAL PART

Experiments at atmospheric pressure. These experiments constitute the major portion of our work. Here all the accessory effects of altitude such as excessive light, temperature changes, increased evaporation, reduced carbon dioxide tension and reduced barometric pressure are eliminated and we have but one factor in common with high altitude, namely, reduced partial pressure of oxygen. The methods of obtaining the particular atmosphere desired for study is described in the previous paper. For purposes of illustration we here give the protocol of one complete and typical experiment; the results in the remaining experiments will be merely summarized.

In all of the tables which follow we have expressed the haemoglobin in grams per 100 cc. of blood. In order to translate this figure into the ordinary clinical scale (100 per cent = normal) the figures given should be multiplied by 7.14.

EFFECT OF AN ATMOSPHERE OF 10 PER CENT OXYGEN AT ATMOSPHERIC PRESSURE

March 21, 1914. Rabbit No. 7. Weight, 1620 g.

Haemoglobin (dilution 1 : 200). Scale: 66.5—12.4 gms. per 100 cc. blood.

Erythrocyte counts:

7,176,000
6,936,000 } Average 6,979,000
6,824,000

Rabbit No. 8. Weight, 1650 g.

Haemoglobin (dilution 1 : 200). Scale 91.1—16.9 gms. per 100 cc. blood.[30]

Erythrocyte counts:

6,304,000
6,280,000
6,686,000 } Average 6,438,000
6,480,000

March 22, 1914.

11.50 a.m. Rabbits placed in chamber. The circulation of air through soda lime and sulphuric acid started. The chamber communicated with the outside air. No oxygen admitted. The animals gradually reduced the oxygen content of the atmosphere in the chamber.

11.30 p.m. Oxygen 13.6 per cent.

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[30] These animals had been previously subjected to another experiment. Rabbit 8 had not returned to normal condition which accounts for the very high concentration of haemoglobin noted.

March 23, 1914.
7.10 a.m. Oxygen turned on. Chamber closed to outside air.
8.30 a.m. Oxygen 9.7 per cent.
10.00 a.m. CO_2, 0.15 per cent.
12.15 p.m. O_2, 10.7 per cent.
8.45 p.m. O_2, 13 per cent.
8.50 p.m. CO_2, 0.15 per cent.
9.30 p.m. Humidity, 23 per cent.
March 24, 1914.
9.30 a.m. O_2, 13.9 per cent.
9.55 a.m. O_2 turned off.
10.00 a.m. CO_2, 0.17 per cent.
11.30 a.m. CO_2, 0.18 per cent. CO_2 absorber changed.
1.20 p.m. O_2, 11.1 per cent; O_2 turned on.
2.30 p.m.–4.30 p.m., O_2 turned off.
8.30 p.m. O_2, 10.2 per cent.
March 25, 1914.
10.20 a.m. O_2, 10.4 per cent.
10.30 a.m. CO_2, 0.07 per cent.
10.50 a.m. CO_2, 0.05 per cent.
11.30 a.m. Humidity, 32.5 per cent.
1.00 p.m. CO_2, 0.05 per cent.
7.40 p.m. O_2, 10.3 per cent.
March 26, 1914.
9.50 a.m. O_2, 10.6 per cent.
10.00 a.m. Humidity, 37.0 per cent.
11.30 a.m. CO_2, 0.13 per cent.
8.40 p.m. O_2, 11.7 per cent.
9.20 p.m. CO_2, 0.1 per cent.
March 27, 1914.
9.40 a.m. O_2, 14.4 per cent.
9.50 a.m. CO_2, 0.23 per cent.
10.00 a.m. O_2 turned off.
11.30 a.m. CO_2, 0.19 per cent.
2.30 p.m. Changed CO_2 absorber.
3.15 p.m. O_2, 12.1 per cent.
7.00 p.m. O_2, 10.6 per cent.
7.10 p.m. CO_2, 0.09 per cent.
7.45 p.m. O_2 turned on.
March 28, 1914.
11.30 a.m. O_2, 10 per cent.
2.15 p.m. CO_2, 0.08 per cent.
2.45 p.m. Humidity, 42 per cent.
7.10 p.m. O_2, 10.2 per cent.
7.25 p.m. CO_2, 0.1 per cent.
March 29, 1914.
10.30 a.m. O_2, 10.3 per cent.
10.45 a.m. CO_2, 0.11 per cent.

11.00 a.m. Humidity, 39 per cent.
6.55 p.m. O_2, 11.3 per cent.
7.10 p.m. CO_2, 0.14 per cent.
March 30, 1914.
2.00 p.m. O_2, 12.9 per cent.
2.00 p.m.–8.00 p.m. O_2 turned off.
4.30 p.m. CO_2, 0.11 per cent.
8.10 p.m. O_2, 10.4 per cent.
March 31, 1914.
1.30 p.m. O_2, 11.2 per cent.
1.40 p.m. CO_2, 0.15 per cent.
2.00 p.m. Rabbits removed from box.

Rabbit No. 7. Weight, 1550 g. Loss of weight, 70 g.

Haemoglobin (dilution 1 : 200). Scale: 92.3 – 17.2 g. per 100 cc. blood. Increase of 4.8 g. = 38.6 per cent.

Erythrocyte counts:

7,752,000
7,824,000 } Average 7,635,000. Increase of 656,000 = 9.4 per cent.
7,328,000

Rabbit No. 8. Weight, 1620 g. Loss of weight 30 g.

Haemoglobin (dilution 1 : 200). Scale: 104.7 – 19.5 g. per 100 cc. blood. Increase of 2.6 g. = 15 per cent.

Erythrocyte counts:

8,696,000
8,560,000 } Average 8,504,000. Increase of 2,066,000 = 32 per cent.
8,256,000

TABLE 1

Control experiments at atmospheric pressure with normal oxygen pressure (20.8 per cent)

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 7...	1580	1560	– 20	6.7	6.8	+1.5	11.6	13.9	+19.8
8...	1300	1500	+200	7.1	7.1	0	14.2	16.6	+16.9
9...	852	970	+118	5.3	5.8	+9.4	10.9	11.6	+ 6.4
10...	861	935	+ 74	6.4	6.5	+1.6	13.1	13.7	+ 4.6
11...	790	898	+108	6.2	5.8	−6.4	12.7	11.5	– 9.4
Dog No. 1...	2140	2080	– 60	5.7	5.9	+3.5	10.3	11.2	+ 8.7
2...	3520	3320	−200	5.4	5.9	+9.3	10.2	12.3	+20.6
Average percentage blood change.....						+2.7			+9.7

The recorded variations in the oxygen were due to marked changes in the temperature of the room which the heat regulating mechanism could not take care of. However, in an experiment of this duration it is very difficult indeed to prevent some variations and we have presented the experiment in detail as a fair average of what we have been able to accomplish with the apparatus without extraordinary care. We have in many experiments kept the oxygen content within very much narrower limits.

TABLE 2

Atmospheric pressure, oxygen 16 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 29...	1740	1480	−260	4.7	4.5	− 4.3	11.0	8.4	−23.6
30...	1420	1610	+190	4.5	4.9	+ 8.8	9.2	10.1	+9.8
31...	1510	1680	+170	5.7	6.4	+12.3	11.8	11.7	−0.8
32...	1400	1500	+100	6.8	6.9	+ 1.5	12.7	13.6	+7.1
Average percentage blood change						+4.6			−2.0

TABLE 3

Atmospheric pressure, oxygen 14 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 30...	1610	1540	− 70	4.9	4.8	− 2.0	10.1	10.8	+ 6.9
31...	1680	1720	+ 40	6.4	6.6	+ 3.1	11.7	14.6	+24.8
32...	1500	1510	+ 10	6.9	6.9	0	13.6	14.0	+ 2.9
39...	1500	1450	− 50	5.0	7.1	+42.0	12.4	14.1	+13.7
40...	1550	1780	+ 30	5.2	7.2	+38.5	11.9	15.6	+31.1
41...	1600	1720	+120	5.7	7.2	+26.3	11.8	15.6	+32.2
42...	1440	1380	− 60	5.3	5.5	+ 3.8	12.0	13.0	+21.5
43...	1620	1560	− 60	5.2	5.7	+ 9.6	10.7	13.0	+21.5
Average percentage blood change						+15.2			+17.4

TABLE 4

Atmospheric pressure, oxygen 12 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 5...	1760	1700	− 60	6.7	8.4	+25.4	14.0	17.0	+21.4
6...	1560	1600	+ 40	8.4	8.5	+ 1.2	14.4	17.4	+20.8
30...	1540	1670	+130	4.8	5.5	+14.6	10.8	12.3	+13.9
31...	1720	1840	+120	6.6	7.1	+ 7.6	14.6	16.3	+11.6
32...	1510	1470	− 40	6.9	8.5	+23.2	14.0	16.3	+16.4
Average percentage blood change						+14.4			+16.8

TABLE 5

Atmospheric pressure, oxygen 10 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 7...	1620	1550	− 70	7.0	7.6	+ 8.6	12.4	17.2	+38.7
8...	1650	1620	− 30	6.4	8.5	+32.8	16.9	19.5	+15.4
9...	970	1060	+ 90	5.8	7.5	+29.3	11.6	15.7	+35.3
10...	935	1070	+135	6.5	7.3	+12.3	13.7	16.4	+19.7
11...	898	973	+ 75	5.8	7.3	+25.9	11.5	17.0	+47.8
12...	1200	1220	+ 20	6.9	7.8	+13.0	14.6	16.6	+13.7
13...	1280	1300	+ 20	6.8	7.1	+ 4.4	12.0	16.2	+35.0
14...	1120	1210	+ 90	6.9	7.2	+ 4.3	13.3	15.6	+17.3
15...	1050	1100	+ 50	5.4	6.6	+22.2	10.6	16.8	+58.5
16...	1010	1095	+ 85	6.0	7.0	+16.7	11.2	15.0	+33.9
22...	1400	1140	−260	6.7	7.1	+ 6.0	14.7	15.9	+ 8.2
23...	1320	1200	−120	5.7	7.6	+33.3	13.8	15.1	+ 9.4
24...	1420	1350	− 70	6.5	7.8	+20.0	13.9	15.6	+12.2
Dog* No. 1...	2420	2460	+ 40	6.6	7.6	+15.2	11.8	13.7	+16.2
2...	3820	3520	−300	6.1	8.2	+34.4	10.2	14.3	+40.2
Average percentage blood change						+18.5			+26.7

* The experiments with dogs 1 and 2 lasted 12 days.

Experiments at low barometric pressure. In these experiments we have eliminated all the factors characteristic of high altitude except reduced atmospheric pressure and the corresponding reduction of the partial pressure of oxygen which this entailed. These experiments were performed in order to determine whether reduced barometric pressure plays any rôle in the polycythaemia of altitude apart from the reduced partial pressure of oxygen. If the effects of reduced barometric pressure are dependent solely on the reduced partial pressure of oxygen then the results should be identical at atmospheric pressure and at reduced pressure when the same partial pressure of oxygen prevails. We shall not present a protocol of one of these experiments because there never occurred any deviation in the pressure or in the composition of the atmosphere of the chamber from that which we desired to main-

TABLE 6

Atmospheric pressure, oxygen No. 19 and No. 20, 9 per cent; No. 21, 8 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 19...	1580	1390	−190	7.1	7.1	0	13.8	14.5	+5.1
20...	1660	1107	−533	7.2	8.3	+15.3	14.1	16.9	+20.0
21...	1150	1165	+15	6.9	8.3	+20.3	13.8	16.9	+22.5
Average percentage blood change						+11.9			+15.9

TABLE 7

Atmospheric pressure, oxygen 6 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 27...	840	630	−210	6.6	8.2	+24.2	12.8	18.6	+45.3
28...	850	560	−290	5.9	6.6	+11.9	11.9	14.9	+25.2
Average percentage blood change						+18.0			+35.2

TABLE 8

Atmospheric pressure, oxygen 10 per cent. Showing progressive blood changes during experiment and the return to normal following experiment

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Dog No. 1			
Before experiment	2320	6.6	11.8
2 day run	2380	6.6 (0)	12.4 (+5.1%)
4 day run	2420	5.9 (−10.6%)	11.3 (−4.2%)
12 day run	2460	7.6 (+15.2%)	13.7 (+16.1%)
8 days after experiment		7.1 (+7.6%)	13.2 (+11.9%)
29 days after experiment		5.9 (−10.6%)	11.6 (−1.7%)
Dog No. 2			
Before experiment	3700	6.1	10.2
2 day run	3820	6.5 (+6.5%)	12.6 (+23.5%)
4 day run	3780	6.5 (+6.5%)	12.8 (+25.3%)
12 day run	3520	8.2 (+34.4%)	14.3 (+40.2%)
8 days after experiment		7.7 (+26.2%)	13.6 (+33.3%)
29 days after experiment		6.4 (+4.9%)	11.7 (+14.7%)
40 days after experiment		6.1 (0)	10.6 (+3.9%)
Rabbit No. 15			
Before experiment	1050	5.4	10.6
3 day run		5.9 (+9.3%)	13.3 (+25.5%)
6 day run	1100	6.6 (+22.2%)	16.8 (+58.5%)
11 day run	1130	5.7 (+5.6%)	15.9 (+50.0%)
Rabbit No. 16			
Before experiment	1010	6.0	11.2
3 day run		6.1 (+1.7%)	12.3 (+9.8%)
6 day run	1095	7.0 (+16.7%)	15.0 (+33.9%)
11 day run	1150	6.9 (+15.0%)	15.3 (+36.6%)

TABLE 9

Atmospheric pressure, oxygen 6 per cent. Showing effect of 2 and 7 day exposure to the atmosphere

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 27			
Before experiment	840	6.6	12.8
2 day run	720	7.2 (+9.1%)	14.7 (+14.8%)
7 day run	630	8.2 (+24.2%)	18.6 (+45.3%)
Rabbit No. 28			
Before experiment	850	5.9	11.9
2 day run	750	6.2 (+5.1%)	13.8 (+16.0%)
7 day run	560	6.6 (+11.9%)	14.9 (+25.2%)

TABLE 10

Atmospheric pressure, oxygen 10 per cent. Showing the persistance of blood effects after experiment

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 7			
Before experiment.....	1620	7.0	12.4
7 day run.....	1550	7.6 (+8.6%)	17.2 (+38.7%)
1 day after experiment		8.0 (+14.3%)	14.8 (+19.4%)
2 days after experiment			15.4 (+24.2%)
3 days after experiment			16.1 (+29.8%)
18 days after experiment			16.2 (+30.6%)
Rabbit No. 8			
Before experiment	1650	6.4	16.9
7 day run.....	1620	8.5 (+32.8%)	19.5 (+15.4%)
1 day after experiment		8.3 (+29.7%)	19.8 (+17.2%)
6 days after experiment		8.5 (+32.8%)	19.5 (+15.4%)
Rabbit No. 9			
Before experiment.....	970	5.8	11.6
7 day run.....	1060	7.5 (+29.3%)	15.7 (+35.3%)
2 days after experiment		7.7 (+32.8%)	16.7 (+44.0%)
10 days after experiment		7.4 (+27.6%)	15.3 (+31.9%)
33 days after experiment		6.0 (+3.4%)	11.8 (+1.0%)
Rabbit No. 10			
Before experiment.....	935	6.5	13.7
7 day run.....	1070	7.3 (+12.3%)	16.4 (+19.7%)
2 days after experiment		7.2 (+10.8%)	15.8 (+15.3%)
10 days after experiment		7.6 (+16.9%)	16.8 (+22.6%)
33 days after experiment	1340	7.5 (+15.4%)	15.8 (+15.3%)
Rabbit No. 11			
Before experiment.....	898	5.8	11.5
7 day run.....	973	7.3 (+25.9%)	17.0 (+47.8%)
2 days after experiment		7.2 (+24.1%)	14.7 (+27.8%)
10 days after experiment		6.9 (+19.0%)	14.8 (+28.7%)
33 days after experiment	1250	6.9 (+19.0%)	14.5 (+26.1%)
Rabbit No. 12			
Before experiment.....	1200	6.9	14.6
7 day run.....	1220	7.8 (+13.0%)	16.6 (+13.7%)
5 days after experiment	1280	6.9 (0)	17.4 (+19.2%)
Rabbit No. 13			
Before experiment.....	1280	6.8	12.0.
7 day run.....	1300	7.1 (+4.4%)	16.2 (+35.0%)
5 days after experiment	1300	6.7 (−1.5%)	17.3 (+44.2%)
Rabbit No. 14			
Before experiment.....	1120	6.9	13.3.
7 day run.....	1210	7.2 (+4.3%)	15.6 (+17.3%)
5 days after experiment	1320	7.1 (+2.9%)	17.2 (+29.3%)

tain. The ventilation of the reduced pressure chamber was from 22 to 24 liters per minute. At this rate of ventilation with two rabbits in the chamber (this number was always employed) the oxygen was always 20.5 per cent and the carbon dioxide 0.2 per cent.

Results. The results can be presented most satisfactorily in tabular form. The experiments are divided into two large groups, (1) those at atmospheric pressure and (2) those at reduced pressure. In these two sets of experiments we have attempted to work at approximately the same pressure of oxygen. We shall first present the larger group of

TABLE 11

Atmospheric pressure, oxygen No. 19 and No. 20, 9 per cent; No. 21, 8 per cent. Showing effect of 7 and 14 day exposures and in No. 21 the post-experimental effect

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 19			
Before experiment	1580	7.1	13.8
7 day run		7.1 (0)	14.5 (+5.1%)
14 day run	1390	7.5 (+5.6%)	16.5 (+19.6%)
Rabbit No. 20			
Before experiment	1660	7.2	14.1
7 day run		8.3 (+15.3%)	16.9 (+19.9%)
14 day run	1107	9.0 (+25.0%)	18.1 (+28.4%)
Rabbit No. 21			
Before experiment	1150	6.9	13.8
7 day run	1165	8.3 (+20.3%)	16.9 (+22.5%)
14 day run	1090	7.9 (+14.5%)	17.0 (+23.2%)
3 days after experiment	1105	8.8 (+27.5%)	19.0 (+37.7%)

TABLE 12

Experiments with white rats. Atmospheric pressure oxygen 10 per cent

Duration of experiments—three weeks

CONTROL RATS IN 20.8 PER CENT OXYGEN		RATS IN 10 PER CENT OXYGEN	
Erythrocytes		*Erythrocytes*	
No. 1	9.1	No. 5	11 4
No. 2	7.4	No. 6	10.7
No. 3	7.8	No. 7	10.1
No. 4	9.8	No. 8	11.3
Average	8.5	Average	10.9

TABLE 12—Continued.

CONTROL RATS IN 20.8 PER CENT OXYGEN		RATS IN 10 PER CENT OXYGEN	
Haemoglobin		*Haemoglobin*	
No. 1	17.1	No. 5	21.2
No. 2	13.7	No. 6	19.3
No. 3	17.0	No. 7	19.8
No. 4	13.6	No. 8	21.5
Average	15.8 g. per 100 cc. blood	Average	20.5 g. per 100 cc. blood

Erythrocytes increased 2.4 million or 28.2 per cent.
Haemoglobin increased 4.7 g. per 100 cc. blood or 29.7 per cent.
Control rats (20.8 per cent oxygen).
No. 11. 7.3 gr. haemoglobin per kilo body weight.
No. 12. 7.8 gr. haemoglobin per kilo body weight.
Average, 7.55 gr. per kilo.
Rats after three weeks in 10 per cent oxygen.
No. 9. 12.5 gr. haemoglobin per kilo body weight.
No. 10. 9.1 gr. haemoblogin per kilo body weight.
Average, 10.8 gr. per kilo.
Increase, 3.25 gr. per kilo or 43 per cent.

TABLE 13

Atmospheric pressure. Showing effect of high content of carbon dioxide with the normal oxygen content (20.5 per cent) of atmosphere. Carbon dioxide No. 105 and No. 106 = 0.5 per cent, No. 17 and No. 18. Carbon dioxide = 1 per cent

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 105	1520	1290	−230	6.5	6.9	+6.2	13.4	13.7	+2.2
No. 106	1460	1360	−100	6.2	6.8	+9.7	13	14.9	+14.6
Average percentage blood change at 0.5% CO_2						+8.0			+8.4
Rabbit No. 17	1300	1410	+110	6.5	7.1	+9.2	12.8	15.0	+17.2
No. 18	930	940	+10	6.1	6.7	+9.8	11.2	12.6	+12.5
Average percentage blood change at 1% CO_2						+9.5			+14.8

experiments, i.e., those at atmospheric pressure. This group of experiments is further subdivided according to the animals used and according to the length of the experiments. Tables are also given showing the post-experimental effects of diminished oxygen pressure and the time required to return to normal. For the purpose of illustrating these various points it will be seen that the same experiments may be recorded in different tables.

Tables 1 to 13 record experiments at atmospheric pressure.

Total haemoglobin determinations were made on four rats of this series.

Tables 14 to 20 record experiments at reduced barometric pressure.

TABLE 14

Reduced barometric pressure. Pressure = 593 mm. Hg. Corresponding oxygen tension, 17 per cent of an atmosphere, equivalent altitude, 2020 m.

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 33...	1800	1560	−240	7.4	7.0	−5.4	13.2	13.8	+4.5
No. 34..	1700	1440	−260	7.2	8.1	+12.5	15.5	14.5	−6.5
Average percentage blood change.....						+3.5			−1.0

TABLE 15

Reduced barometric pressure. Pressure = 495 mm. Hg. Corresponding oxygen tension, 14 per cent of an atmosphere, equivalent altitude 3,500 m.

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 33...	1560	1610	+50	7.0	7.2	+2.9	13.8	14.1	+2.2
No. 34..	1440	1520	+80	8.1	8.1	0	14.5	14.9	+2.8
No. 35...	1760	1700	−60	5.0	5.5	+10.0	11.5	12.6	+9.6
No. 36...	1690	1640	−50	5.6	4.6	−17.9	9.7	9.7	0
No. 44...	1520	1440	−80	5.8	6.7	+15.5	11.0	13.3	+20.9
No. 45...	1420	1410	−10	5.3	6.5	+22.6	12.1	13.5	+11.6
Average percentage blood change.....						+5.6			+7.8

DISCUSSION OF RESULTS

The results shown in tables 1 to 6 clearly prove that great increases in erythrocytes occur when dogs, rabbits or rats are kept in an atmosphere of low oxygen concentration even at atmospheric pressure. When the atmosphere of the chamber contained the normal concentration of oxygen (20.8 per cent) or even 16 per cent oxygen the changes in the blood count were insignificant. When the oxygen is reduced to 14 per cent marked increases in the blood count occur within one week. The increases noted at 14 per cent and 12 per cent oxygen in our

TABLE 16

Reduced barometric pressure. Pressure 422 mm. Hg. Corresponding oxygen tension 12 per cent of an atmosphere, equivalent altitude, 4730 m.

Duration of experiment—one week

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 37...	1860	1780	−80	5.2	6.0	+15.4	8.9	12.6	+41.6
No. 38...	1760	1640	−120	5.3	6.8	+28.3	8.3	14.0	+68.7
Average percentage blood change						+21.8			+55.1

TABLE 17

Reduced barometric pressure. Pressure 352 mm. Hg. Corresponding oxygen tension, 10 per cent of an atmosphere, equivalent altitude, 6060 m.

Duration of experiment No. 48 and No. 49 four days; No. 50 and No. 51 five days

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 48...	1950	1640	−310	4.6	4.5	−2.2	6.9	9.8	+42.0
No. 49...	1830	1620	−210	6.3	6.5	+3.2	13.2	15.0	+13.6
No. 50...	1370	1330	−40	7.5	8.7	+16.0	14.9	19.8	+32.9
No. 51...	1420	1380	−40	7.5	8.1	+8.0	14.5	17.3	+19.3
Average percentage blood change.....						+6.2			+27.0

series are practically identical. At 10 per cent oxygen the increases reach the maximum, 18 per cent increase in erythrocytes and 26.7 per cent increase in haemoglobin. The number of experiments at 9 per cent, 8 per cent, and 6 per cent oxygen are too few in number to conclude what the average increase in a large number of animals would be. The variations in individuals in their reaction to atmospheres low in oxygen is so great that large numbers of experiments must be performed in order to arrive at a reliable average figure for a given oxygen concentration. It is to be noted that anaemic animals react more markedly to a

TABLE 18

Reduced barometric pressure. Pressure = 352 mm. Hg. Corresponding oxygen tension 10 per cent of an atmosphere, equivalent altitude, 6060 m.

Duration of experiment—one day

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 46...	1800	1720	−80	5.9	6.6	+11.9	13.6	14.1	+3.7
No. 47...	2020	1910	−110	6.3	6.5	+3.2	13.8	13.9	+0.7
No. 48...	1950	1760	−190	4.6	4.6	0	6.9	7.6	+10.0
No. 49...	1830	1700	−130	6.3	6.3	0	13.2	13.4	+1.5
No. 50...	1370	1370	0	7.5	7.4	−1.3	14.9	14.9	0
No. 51...	1420	1440	+20	7.5	7.7	+2.7	14.5	14.7	+1.4
Average percentage blood change.....						+2.7			+2.9

TABLE 19

Reduced barometric pressure. Pressure = 352 mm. Hg. Corresponding oxygen tension 10 per cent of an atmosphere equivalent altitude 6060 m.

Duration of experiment—two days

ANIMAL	WEIGHT			ERYTHROCYTES MILLION PER CMM.			HAEMOGLOBIN GR. PER 100 CC. BLOOD		
	Before	After	Change	Before	After	Change	Before	After	Change
			grams			*per cent*			*per cent*
Rabbit No. 46...	1800	1720	−80	5.9	6.5	+10.2	13.6	14.1	+3.7
No. 47...	2020	1890	−130	6.3	6.8	+7.9	13.8	14.9	+8.0
No. 48...	1950	1690	−260	4.6	4.3	−6.5	6.9	7.6	+10.0
No. 49...	1830	1660	−170	6.3	6.2	−1.6	13.2	13.7	+3.8
No. 50...	1370	1320	−50	7.5	7.5	0	14.9	15.1	+1.3
No. 51...	1420	1420	0	7.5	7.5	0	14.5	15.6	+7.6
Average percentage blood change.....						+1.7			+5.7

reduction in the partial pressure of oxygen, an observation in keeping with the results of David.[31] It would seem, however, from the limited number of experiments performed that the maximum increases in the blood count occur when the oxygen concentration is near 10 per cent.

TABLE 20

Reduced barometric pressure. Pressure 352 mm. Hg. Corresponding oxygen tension 10 per cent of an atmosphere, equivalent altitude, 6060 m.

ANIMAL	WEIGHT	ERYTHROCYTES MILLION PER CMM.	HAEMOGLOBIN GR. PER 100 CC. BLOOD
Rabbit No. 46			
Before experiment.....	1800	5.9	13.6
1 day run.....	1720	6.6 (+11.9%)	14.1 (+3.7%)
2 day run.....	1720	6.5 (+10.2%)	14.1 (+3.7%)
Rabbit No. 47			
Before experiment.....	2020	6.3	13.8
1 day run.....	1910	6.5 (+3.2%)	13.9 (+0.7%)
2 day run.....	1890	6.8 (+7.9%)	14.9 (+8.0%)
Rabbit No. 48			
Before experiment.....	1950	4.6	6.9
1 day run.....	1760	4.6 (0)	7.6 (+10.0%)
2 day run.....	1690	4.3 (−6.5%)	7.6 (+10.0%)
4 day run.....	1640	4.5 (−2.2%)	9.8 (+42.0%)
Rabbit No. 49			
Before experiment.....	1830	6.3	13.2
1 day run.....	1700	6.3 (0)	13.4 (+1.5%)
2 day run.....	1660	6.2 (−1.6%)	13.7 (+3.8%)
4 day run.....	1620	6.5 (+3.2%)	15.0 (+13.6%)
Rabbit No. 50.....			
Before experiment.....	1370	7.5	14.9
1 day run.....	1370	7.4 (−1.3%)	14.9 (0)
2 day run.....	1320	7.5 (0)	15.1 (+1.3%)
5 day run.....	1330	8.7 (+16.0%)	19.8 (+32.9%)
Rabbit No. 51			
Before experiment.....	1420	7.5	14.5
1 day run.....	1440	7.7 (+2.7%)	14.7 (+1.4%)
2 day run.....	1420	7.5 (0)	15.6 (+7.6%)
5 day run.....	1380	8.1 (+8.0%)	17.3 (+19.3%)

It is very interesting that increases in the blood count occur even with oxygen concentrations at the lowest level at which it is possible to keep an animal continuously for a week in fairly good condition. It has been shown previously in this laboratory that the stimulation of the medul-

[31] David Deutsch. Arch. f. klin. Med., 1913, cix, 129.

lary centers by decreased oxygen fixation passes into depression and paralysis when oxygen fixation falls below a certain level. The work here reported indicates that the bone marrow differs in this regard from the medullary centers. Since approximately a week is required for stimulation of the bone marrow to become evident it would seem that the nervous system would succumb before oxygen fixation by the bone marrow could be reduced to the point of depression. The relative effect of different concentrations of oxygen at atmospheric pressure is shown in the following table:

TABLE 21

OXYGEN	NUMBER OF EXPERIMENTS	BLOOD CHANGE	
		Erythrocytes	Haemoglobin
per cent		*per cent*	*per cent*
20	7	2.7	9.7
16	4	4.6	−1.9
14	8	15.2	17.4
12	5	14.4	16.8
10	15	18.5	26.7
(9, 8, and 6)	5	14.3	29.0

The results prove beyond doubt that a decrease in the partial pressure of the oxygen of the respired air causes an increase in the erythrocytes and haemoglobin per unit volume of blood and that the increase occurs even at atmospheric pressure under conditions which eliminate all of the physical effects of decreased barometric pressure and high mountain climates.

If a sufficiently large number of experiments had been performed at each oxygen concentration the irregularities in the above table would in all probability have disappeared. In this case it probably would have been noted that the effect of lowering the oxygen tension would have increased more or less regularly after 14 per cent oxygen had been reached until the optimum, about 10 per cent, had been attained. This would have involved a large expenditure of time and energy which in the presence of the above results was not deemed necessary.

Tables 14 to 17 show the effect of corresponding partial pressures of oxygen obtained by reducing the barometric pressure. The results are summarized in the following table:

TABLE 22

BAROMETRIC PRESSURE MM. HG.	NUMBER OF EXPERIMENTS	PARTIAL PRESSURE OF OXYGEN IN PER CENT OF AN ATMOSPHERE	BLOOD CHANGE	
			Erythrocytes	Haemoglobin
593	2	17	+3.5	−1.0
495	6	14	+5.6	+7.8
422	2	12	+21.8	+55.1
352*	4*	10*	+6.2*	+27.0*

* These experiments only lasted four to five days whereas the others were of a week's duration.

It will be noted on examining tables 14 to 17 that the average figures given above were obtained from relatively few experiments and therefore merely indicate the general trend of what average results might have been obtained if a larger number of individuals had been used. Thus in the work at 12 per cent oxygen only two animals were used. We regard it as highly improbable that in a large number of experiments animals would show as marked an increase in the blood count at 12 per cent as at 10 per cent oxygen. Unfortunately the experiments at 10 per cent oxygen were not allowed to proceed a week as in the other cases but were interrupted at the end of four or five days. This was due to the fact that at the time the experiments were performed other phases of the subject were under consideration. On comparing tables 20 and 21 it will be seen that the increase in the blood count at reduced oxygen tension occurs whether the partial pressure of the oxygen is reduced by diluting the oxygen at atmospheric pressure with the inert gases of the atmosphere or by reducing the barometric pressure. The general trend of the results in both cases is the same and we believe that if a sufficiently large number of observations were made there would be no difference.

The question then presents itself whether the increased blood counts noted are to be attributed to concentration of the blood, i.e., a reduction in the total blood volume, to changes in the distribution of the erythrocytes or to increased production of erythrocytes and haemoglobin by the bone marrow. The fact that the increase in blood count is noted at atmospheric pressure when the partial oxygen pressure is lowered even with a humidity which is equal to or greater than that of the normal atmosphere speaks strongly against increased evaporation of water as the mechanism by which any increase in the concentration of the blood could occur. Furthermore, a study of the weight of the

animals before and after experiments proves conclusively that the increased blood counts per unit volume of blood cannot be due to increased evaporation of water from the animals. In many cases the animals gained in weight during the experiment where marked increase in the count was noted. In other cases the animals showed marked loss of weight without any change in the count. The blood changes were entirely independent of any changes in the weight of the animals, as the tables definitely show.

In order to determine whether there was an alteration in the distribution of the erythrocytes and plasma in the large and small vessels as suggested by Foa[32] we have made counts of blood obtained from the marginal ear vein and then of blood from the carotid and heart. In all cases the counts were the same within the limits of experimental error. The only probable explanation remaining is that the increased blood counts are due to increased activity of the bone marrow. As further evidence in favor of this view we present the following observations: (1) We found in our work on the white rat, Table 12, that the animals kept for three weeks in an atmosphere of 10 per cent oxygen at atmospheric pressure possess on the average 43 per cent more haemoglobin per kilo body weight than the animals kept in the ordinary atmosphere. These findings are in keeping with the results of Jaquet and Suter[33] and Abderhalden[34] who made similar determinations. (2) Blood smears from animals showing increased blood counts after exposure to atmospheres poor in oxygen when stained with Jenner stain show a number of basophilic erythrocytes. This staining reaction is characteristic of newly formed erythrocytes. Many of these basophilic erythrocytes were abnormally large especially in those animals which showed a marked increase in the haemoglobin without very much change in the number of erythrocytes. This probably explains the increase in the amount of haemoglobin per corpuscle in certain cases. (3) Examination of the bone marrow showed macroscopically a disappearance of the fat and an extension of the red marrow and microscopically a marked hyperplasia of the erythrocyte forming centers and an equally marked dilatation of the capillaries and small vessels of the marrow which were engorged with blood. The picture is practically identi-

[32] Foa: Laborat. scient. internat. du Mont Rosa. Trav. de l'anée 1903, Turin 1904 cited from Zuntz, Loewy, Müller and Caspari, "Hohenklima und Bergwanderungen," 1906.

[33] Jaquet and Suter: Correspondenzblt. f. Schweizer Aerzte, 1898, xxviii, 104.

[34] Abderhalden: Zeitsch. f. Biol., 1902, xliii, 123.

cal with that seen after haemorrhage except for the engorgement of the vessels which is not seen in the latter condition. We are greatly indebted to Dr. C. H. Bunting for the microscopical examination of the blood smears, bone marrow and other organs of our animals and we desire herewith to extend our thanks to him and to acknowledge his valuable assistance.

The relation between the increase in the number of erythrocytes and haemoglobin noted in our experiments is interesting. Oliver[35] working at Arosa and Davos found that the erythrocytes increase 10 per cent and the haemoglobin 5 per cent. Roemisch[36] found at Arosa that the increase in the haemoglobin was about half as great as the increase in erythrocytes. Van Voornveld[37] states that most investigators agree that the erythrocytes increase more rapidly than the haemoglobin. Abderhalden found that the increase in erythrocytes is always proportional to the increase in haemoglobin. Schaumann and Rosenqvist[38] find that the increase in haemoglobin lags behind that in erythrocytes. Eggers[39] found in two people there occurred a much smaller increase in erythrocytes than in haemoglobin. Bürker and his co-workers[40] likewise found that in two persons investigated the haemoglobin showed a greater increase than the erythrocytes. In their work on artificial pneumothorax, Bürker, Ederle and Kircher[41] found that the erythrocyte count increased more rapidly and showed a greater increase than the haemoglobin in the one animal of which they publish the details of the experiment. Nasmith and Harrison[42] state that animals chronically poisoned with carbon monoxide show a relatively greater increase in erythrocytes than in haemoglobin.

Under the conditions obtaining in our work the haemoglobin showed on the average a much greater increase than the erythrocytes in response to decreased partial pressure of oxygen both at atmospheric and at reduced barometric pressure. There are, however, many individual cases where the increase in the erythrocytes is greater than that of the haemoglobin as may be seen from the tables. These cases are in the

[35] Oliver: "A contribution to the study of the blood and blood pressure," London, 1901.

[36] Roemisch: Cited from Van Voornveld, q. v.

[37] Van Voornveld: Arch. f. d. gesammt. Physiol., 1902, xcii, 1.

[38] Schaumann and Rosenqvist: Zeitschr. f. klin. Med., 1898, xxxv, 126, 315.

[39] Eggers: Arch. f. exp. Path. u. Pharm., 1897, xxxix, 426.

[40] Bürker, Jooss, Moll and Neumann: Zeitsch. f. Biol., 1913, lxi, 379.

[41] Bürker, Ederle and Kircher: Zentralblt. f. Physiol., 1913, xxvii, p. 623.

[42] Loc. cit.

minority, however. In some cases as in Rabbit 7, table 5, the increase in haemoglobin was so great compared with the increase in the erythrocytes that we examined the plasma for haemoglobin, but never found any haemoglobin outside of the corpuscles. Therefore in most of our experiments there was found an increase in the amount of haemoglobin per corpuscle. The greatest increase in the haemoglobin per corpuscle in the experiments at atmospheric pressure was 30 per cent, noted in Rabbit 15, table 5. The greatest decrease in haemoglobin per corpuscle was 20 per cent noted in Rabbit 39, table 3. The effect of reduced oxygen pressure on the color index is therefore very variable.

The time required for the increased blood count to develop is of much interest. Investigations of the effect of high altitude on the blood count do not agree entirely as to the time of onset of the blood change. Ehrlich and Lazarus[43] state that the increase occurs immediately on reaching a place of considerable altitude. Oliver[44] states that the increase occurs soon after arival at high altitude, being apparent within twenty-four hours. Abderhalden[45] found that the erythrocytes and haemoglobin increased within a few hours. Douglas, Haldane, Henderson and Schneider[46] found that erythrocytes and haemoglobin increase during the first few days and continue to increase for several weeks on Pike's Peak. Schneider and Havens[47] find that a rapid increase in erythrocytes and haemoglobin in the peripheral vessels occurs during the first two to four days of residence at high altitude. Our results on this point at atmospheric pressure are shown in tables 8 and 9 and at reduced barometric pressure in tables 18, 19, and 20. From tables 8 and 9 it will be seen that all of the animals except Dog 1 and Rabbit 16 show definite increase in the blood counts at the time of the first observations, viz., after two or three days. On the other hand, at low barometric pressure corresponding to 10 per cent oxygen, observations on six rabbits showed no increase after twenty-four or forty-eight hours' exposure to the atmosphere. Our observations were not made on a sufficiently large number of animals to admit of generalization. In no case, however, were the maximum counts obtained in less than one week.

[43] Ehrlich and Lazarus: Anaemia, Nothnagel's Encyclopedia, Philadelphia and London, 1905, p. 22.

[44] Loc. cit.

[45] Loc. cit.

[46] Loc. cit.

[47] Schneider and Havens: This Journal, 1915, xxxvi, 380.

A few experiments were performed to determine whether a longer exposure to the atmospheres poor in oxygen would result in further increasing the blood count. These experiments in which animals were exposed for periods longer than one week to atmospheres low in oxygen indicated that the maximal count is not reached within one week. (Table 11.) This is in agreement with the results of work at high altitude.

In regard to the post-experimental blood changes our results are also in perfect accord with the work of many investigators on the effect of high altitude. Following the removal of animals from the respiratory chamber into the ordinary atmosphere there was often noted a further increase in the blood counts during the first and second day (Table 10). Following this period the erythrocytes and haemoglobin very gradually return to normal often requiring more than a month to reach the level obtaining before the exposure to atmospheres of low oxygen. This slow return to the normal has been noted by all workers at high altitude and the fact that the same effects are noted at normal barometric pressure when the partial pressure of oxygen alone is reduced is striking evidence of the identity of the blood changes under the two conditions and leads to the conclusion that the blood changes noted at high altitude are due essentially to the low partial pressure of oxygen.

Mechanism of the stimulation of the bone marrow. All of the facts presented in the foregoing part of this communication lead to the conclusion that the increase in erythrocytes and haemoglobin at high altitude and in atmospheres of low partial pressure of oxygen whether at atmospheric pressure or at reduced barometric pressure is due to stimulation of the bone marrow. The question of the mechanism of the stimulation then presents itself. Bert looked upon the increase in the oxygen capacity of the blood at high altitude as constituting an important factor in acclimatization to an atmosphere of reduced oxygen tension. Viault, Müntz, and other workers agreed with Bert. The statement that it is the principal factor in acclimatization is not an explanation of the mechanism by which it occurs. A review of the literature since the early work shows clearly that investigators were loathe to accept the increase in the oxygen capacity of the blood at high altitude as a fact or in the face of excellent evidence they sought to explain it as purely a relative increase without any physiological significance. What was the reason for this attitude on the part of investigators? It would seem that the statement, "when the oxygen tension of the air is decreased the oxygen capacity of the blood is in-

creased" was regarded as too teleological to be true. Miescher,[48] however, accepted the proposition as proved and sought to interpret the mechanism of the reaction. He believed that there exists in the bone marrow normally a condition of relative oxygen want which maintains the bone marrow in a condition of activity so that erythrocytes and haemoglobin are being produced constantly at a certain rate. He believed that any further reduction of the oxygen supply to the bone marrow increases this activity. He sought other biological parallels to this stimulation by oxygen want and referred to the increased production of alcohol by yeast under anaerobic conditions. Our views agree closely with those of Miescher. We feel that the parallelism with the activity of yeast which Miescher tried to draw was not the happiest one which could be found. It had been long known that the respiratory center responded with increased functional activity to oxygen want. Furthermore, oxygen want or any other method of reducing oxygen fixation has since been shown to stimulate the respiratory, vaso-constrictor and cardio-inhibitory centers, and also the motor cortex. We have therefore abundant analogy for the stimulation of the bone marrow by decreased oxygen supply to the bone marrow. Stimulation by decreased oxygen fixation and the mechanism by which it may be conceived to be brought about have been discussed by Gasser and Loevenhart[49] and by Loevenhart.[50] The external respiration, the circulation and the bone marrow are the three physiological mechanisms which maintain the chemical environment essential for tissue respiration. The respiratory movements are controlled by the respiratory center, the oxygen capacity of the blood is controlled certainly in large part by the activity of the bone marrow. It is therefore interesting to compare the reactions of the respiratory center and the bone marrow to alterations in the oxygen and carbon dioxide content of the blood as well as to other conditions altering oxidations in the body. Gasser and Loevenhart have shown that the injection of threshhold doses of sodium cyanide or carbon monoxide affect the respiratory center before any other function is measurably affected. This indicated that the cells of the respiratory center are more sensitive to decreased oxygen fixation than any other cells in the body so far as could be determined. It was shown by Haldane and Priestley[51] that the oxygen of the respired air must fall

[48] Miescher: Correspondenzbl. f. Schweizer Aerzte, 1893, xxiii, 809.
[49] Gasser and Loevenhart: Loc. cit.
[50] Loevenhart: Arch. Internal Med., 1915, xv, 1059.
[51] Haldane and Priestly: Journ. Physiol., 1905, xxxii, 225.

to 13 per cent of an atmosphere before the respiratory center is stimulated. It will be seen from our work that we have observed no stimulation of the bone marrow when the oxygen of the respired air falls to 16 per cent. At 14 per cent oxygen we noted stimulation of the bone marrow. At high altitude, however, a decrease of the oxygen pressure of much smaller magnitude has been found to stimulate the bone marrow. It may be that in these cases other factors than oxygen tension play a rôle since in our experiments both at atmospheric and at reduced barometric pressure stimulation was not observed until the oxygen tension reached 14 per cent of an atmosphere. It would seem, therefore, that the bone marrow is perhaps a trifle more sensitive to a decrease in the oxygen supply than the respiratory center. This may be due to the fact that the respiratory center must respond quickly or not at all to decreased oxygen fixation and that it adapts itself more quickly to the new conditions for oxidation than does the bone marrow. In fact it would seem that the bone marrow has no power to adapt itself to decreased oxygen supply since the increased rate of erythrocyte and haemoglobin formation continue indefinitely for a given oxygen tension. It would seem as though the oxygen of the respired air would have to fall to near 14 per cent before the percentage saturation of the haemoglobin with oxygen is appreciably reduced. The respiratory center or the bone marrow could respond only to a decrease in the oxygen of the respired air if this were sufficient to alter their supply of oxygen. It would seem that while the bone marrow is a trifle more sensitive than the respiratory center, the two are however approximately equally sensitive to a decreased supply of oxygen. Grove and Loevenhart and Gasser and Loevenhart have shown that if oxygen fixation by the respiratory center is reduced below a certain level there results a temporary depression or paralysis of the respiratory center. We have made an attempt to determine whether the bone marrow may be likewise depressed. Exposure of animals for a week to an atmosphere of 6 per cent oxygen failed to show any depression of the bone marrow. In fact it was stimulated. It seemed impractical therefore to demonstrate depression of the bone marrow by decreasing oxygen fixation. At such low concentrations of oxygen the animals refuse to eat and conditions became too abnormal in experiments of a week's duration to admit of a clear cut interpretation and the attempt was abandoned. A comparison of the reaction of the respiratory center and the bone marrow to an increase in the carbon dioxide is likewise of considerable interest. It is our belief that the mechanism of this stimulation is

to be found in the acid properties of carbon dioxide and its consequent power to decrease oxygen fixation. In terms of this theory we would say that the velocity of oxygen fixation (R-processes) of the respiratory center is extremely sensitive to carbon dioxide or in other words, to the hydrogen ion concentration. We performed a few experiments (table 13) to determine how sensitive the bone marrow is to an increase in the carbon dioxide. We found definite but slight stimulation of the bone marrow by concentrations of 0.5 per cent to 1 per cent carbon dioxide in the respired air. The bone marrow is therefore far less sensitive to carbon dioxide than the respiratory center. Expressed in terms of our theory therefore, the velocity of oxygen fixation (R-processes) is relatively far less sensitive to carbon dioxide or to the hydrogen ion concentration in case of the bone marrow than in that of the respiratory center. The relative effectiveness of oxygen want and excess of carbon dioxide in altering the processes of oxidation and causing stimulation not only varies in the different tissues of the same individual or species but probably also varies in the same tissue of different species. Thus it would appear that the respiratory center of diving birds is not stimulated by an increase of carbon dioxide, but responds readily to oxygen want. In all cases we believe that the stimulation by oxygen want, excess of carbon dioxide, hydrocyanic acid and many other means is to be attributed to a primary decrease in oxygen fixation or in other words to a primary decrease in the velocity of the R-processes, those oxidative processes which are characteristic of rest and recuperation. The R-processes of different tissues vary greatly in their relative sensitiveness to decreased supply of oxygen and excess of carbon dioxide. The work here presented is in keeping with the theoretical views previously published from this laboratory relative to the A and R processes and the inverse relationship existing between oxygen fixation and functional activity.

CONCLUSIONS AND SUMMARY

1. A decrease in the oxygen tension of the respired air obtained by decreasing the oxygen concentration at atmospheric pressure or by reducing the barometric pressure stimulates the bone marrow and increases the erythrocytes and haemoglobin in the circulating blood in rabbits, white rats and dogs. From five to seven days is required for the increase in the blood count to become very marked but the maximum increase requires a longer exposure.

2. The increase in the erythrocytes and haemoglobin is absolute and not relative. We have been able to increase the total haemoglobin per kilo in rats 43 per cent.

3. In order to produce these effects the oxygen pressure in the respired air must be reduced at least to approximately 14 per cent of an atmosphere.

4. The optimum oxygen pressure for increasing the oxygen capacity of the blood is apparently not far from 10 per cent of an atmosphere.

5. We have not been able to produce depression of the bone marrow by decreasing the oxygen supply. Stimulation results even when the oxygen pressure falls to 6 per cent of an atmosphere.

6. It is possible to stimulate the bone marrow to a certain extent by increasing the carbon dioxide tension of the respired air but it is not an efficient stimulus.

7. A comparison of the respiratory center and the bone marrow in their reaction to a decreased supply of oxygen and an excess of carbon dioxide shows that they are practically equally sensitive to oxygen want but that the respiratory center is far more sensitive to carbon dioxide than is the bone marrow.

8. Our work indicates that the increase in erythrocytes and haemoglobin noted by practically all workers at high altitudes is due largely if not entirely to the decreased partial pressure of oxygen at high altitude, but the very rapid increases in the blood counts noted in man at high altitude and also the increases noted at comparatively very slight elevations is not explained by our work.

9. The physiological significance of the increase in the oxygen capacity of the blood when an atmosphere of low oxygen tension is respired is sufficiently obvious to require no comment.

10. The stimulation shown by the bone marrow in response to decreased oxygen supply is by no means unique. Similar reactions are to be seen in the respiratory, vaso-constrictor and cardio-inhibitory centers and the mechanism is in all cases probably identical. The work is entirely in keeping with the views previously published from this laboratory in regard to the probable mechanism of stimulati n by oxygen want that there is inverse relation between changes in the rate of oxygen fixation and functional activity.

THE STIMULATION OF THE HYPOPHYSIS IN DOGS

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A. INTRODUCTION AND LITERATURE

Attempts to produce glycosuria by the injections of extracts of the hypothesis were reported by Borchardt[1] in 1908. He found that subcutaneous injections of 2 to 4 glands in rabbits regularly produced glycosuria. In dogs his results were inconclusive. Out of 7 animals 2 failed to give sugar in the urine, and in the other cases extracts of 20 to 30 glands were injected. The blood of 2 rabbits showed hyperglycaemia. The glycosuria was transitory, coming on three hours after the injections and rarely lasted through the succeeding twenty-four hours. Rossi[2] reports glycosuria in dogs following the injections of hypophyseal extracts.

Franchinini[3] attempted to repeat Borchardt's results using the latter's technique for the preparation of the extracts. For his experiments he used 22 rabbits, and in only 2 of these was he able to produce glycosuria, which appeared towards the end of life. Autopsies on these animals showed ulcerations and haemorrhages in the duodenum and the small intestines. In his criticism of the positive findings of Rossi, he calls attention to Rossi's use of 0.5 per cent phenol for a preservative, a substance which has been shown by Bukowski[4] to cause glycosuria. He is inclined to believe that there is no specific action in the extracts, but whatever glycosuria may result is attributable to the lesions in the intesinal tract. In support of this view he cites two cases[5] of similar lesions in man, produced by swallowing aqua regia and sodium hydroxide, attended by a glycosuria preceding death. Franchinini cites a

[1] Borchardt, L.: Zeitschr. f. Klin. Med., 1908, lxvi, 332.

[2] Rossi: quoted by Franchinini, Loc. cit. Il Tommasi, 1909, No. 25, 26. 592.

[3] Franchinini: G. Berl. Klin. Wochenschr., 1910, xlvii, 670.

[4] Quoted in Borchardt's article.

[5] Zak, E.: Wiener Klin. Wochenschr., 1908, No. 3, p. 82.

short note by Pal,[6] who agrees with him in getting negative results with hypophyseal extracts.

Goetsch, Cushing, and Jacobsen[7] were able to confirm Borchardt as to the production of glycosuria in rabbits. They also found that injections of posterior lobe extract lowered the tolerance of dogs for cane sugar, but no data is offered on the production of glycosuria in these animals on a normal mixed diet. Following surgical manipulations which involved the crushing of the stalk, as in total hypophysectomy, a transient glycosuria resulted; if however a clean-cut posterior lobe enucleation was performed no sugar appeared. This latter result was one which grew out of a series of hypophysectomies by Crowe, Cushing, and Homans,[8] and appeared to be so constant in their experience that no specific data is presented. In studying the presence of the infundibular lobe secretion in the cerebro-spinal fluid Cushing and Goetsch[9] placed a silver clip on the stalk of the gland in three cases. Two of these showed glycosuria.

Weed, Cushing, and Jacobsen[10] in pursuance of their studies into the control of the hypophysis in carbohydrate metabolism, attacked the problem by hypophyseal puncture, by stimulation of the gland and the superior cervical ganglion electrically. The hypophyseal puncture in rabbits gave glycosuria, a result also obtained by a typical Bernard puncture, even after section of the cord at the level of the fourth thoracic vertebra. In the latter case it was found necessary to allow a sufficient time for the re-accumulation of hepatic glycogen. Stimulation of the superior cervical ganglion (rabbits) and of the gland (cats), after transection of the cord and the reaccumulation of glycogen caused "a prompt and outspoken glycosuria." These results fail if the posterior lobe has been removed from the animal. Rabens and Lifschitz[11] repeated the stimulation of the superior cervical ganglion without an anaesthetic, and estimated the reducing power of the blood, with entirely negative results. They called attention to the lack of published controls of Cushing and associates, and suggested that their results might be attributed to the anaesthesia.

[6] Pal: Semaine Medicale, 1909, xxvi, 312.

[7] Goetsch, E., Cushing, H., and Jacobsen, C.: Johns Hopkins Hospital Bull., 1911, xxii, 165.

[8] Crowe, S. J., Cushing, H., and Homans, J.: Ibid., 1910, xxi, 127.

[9] Cushing, H., and Goetsch, E.: This Journal, 1910, xxvii, 60.

[10] Weed, L. H., Cushing, H., and Jacobsen, C.: Johns Hopkins Hospital Bull., 1913, xxiv, 40.

[11] Rabens, I., and Lifschitz, J.: This Journal, 1915, xxxvi, 47.

As to the cause of the glycosuria, Goetsch, Cushing, and Jacobsen state "We assume that the spontaneous glycosuria represents a hyperglycaemia from the discharge of stored glycogen, which has been set free by the introduction into the circulation of the posterior lobe secretion." Following this and in the work of Weed, Cushing, and Jacobsen, the reader gains the impression that such an assumption has been fully established and adequately proven. The lowering of the tolerance of dogs for cane sugar by injections of posterior lobe extracts, the necessity of "available glycogen" for the production of glycosuria on stimulating the gland, and the two cases of hyperglycaemia in rabbits (Borchardt) renders highly probable indeed the assumption, but it does not constitute final proof.

It seemed to us highly important to extend if possible the results of Weed, Cushing, and Jacobsen on stimulation of the gland to dogs, since their stores of glycogen are not so mobile as those of the cat and rabbit. We felt that by estimating the reducing power of the blood we had a more delicate method of following the effects of stimulation of the gland, and a means of determining finally the cause of the glycosuria, whether it is due to a true hyperglycaemia, or a lowered threshold for sugar presented by the animal under this experimental condition.

B. EXPERIMENTAL METHODS

Anaesthetic

Any experimental study of the sugar in the blood or urine, which involves the use of an anaesthetic demands the most rigorous set of controls. Macleod[12] found that the stimulation of the central end of the vagus nerve of the dog and rabbit under ether anaesthesia led to hyperglycaemia and glycosuria. If these same operations were repeated with oxygen insufflation no change in the sugar level resulted. In his discussion of these results he has to say,

> Although at first sight these results would seem to indicate that the vagus cannot carry afferent fibers to the glycogenic center; this conclusion is not inevitable for it is possible that with the over-arterialization of the blood a small increase in the amount of sugar delivered into it would not cause hyperglycaemia, because of the excess of sugar being burnt up.

He has further noted that oxygen insufflation diminishes the hyperglycaemia following the stimulation of the splanchnic nerve, but it

[12] Macleod, J. J. R.: Diabetes. Longmans and Co., 1913, p. 61, 79.

does not abolish it entirely. Upon this basis he has opposed the use of an anaesthesia, which involves positive ventilation of the lungs, wherever it could be avoided.

More recently Shaffer and Hubbard[13] using the artificial respiration apparatus designed by Gesell and Erlanger[14] have studied the reducing power of the blood, and find that remarkably low levels can be maintained during operative procedure lasting even for several hours. They strongly urge the advantage of working at these lower levels, and suggest that forced respiration be established in all cases, where variations of the blood sugar are being observed. It seemed necessary to adopt some method of ventilating the lungs in the administration of the anaesthetic, since stimulation was to be applied to different points of the brain, a procedure most likely at some time to result in the alteration of the respiratory rhythm. Our method consisted in insufflation with a small continuous air current, led through a tube terminating in the lower cervical trachea. By adjusting the stream carefully the normal spontaneous respiratory rhythm of the animal could be maintained. The cervical location of the tube avoids the possible depressor effects on blood pressure that might supervene in a thoracic location, as shown by Janeway and Ewing.[15]

There is a variable rise in the reducing power of the blood in putting an animal under ether anaesthesia. Our experience has led us to the conclusion, that of the two factors involved, asphyxia, due to the methods of administration of the ether, holding of the breath of the animal, and excitement, the former is much the more important. With an open towel administration of the anaesthetic, attention to the tongue and mucous in the throat, the experiment can be started with a level of 0.10 per cent to 0.13 per cent reducing substances figured to dextrose. Faulty manipulation can just as readily result in beginning with a 0.18 per cent to 0.2 per cent level. Preliminary experimentation with our method of anaesthesia soon showed us that the reducing substances in the blood did not increase with two to three hours of ether administration, but as a rule fell. It is not necessary to quote extensively data from these experiments, since the large number of cases unattended by a rise cited in the body of the paper establish the method as satisfactory. However two experiments with strychnine, which

[13] Shaffer, P. A., and Hubbard, R. S.: Proc. Amer. Soc. Biol. Chem., 1914, iii, 31.

[14] Gesell, R. A., and Erlanger, J.: This Journal, 1914, xxxiii, p. xxxiii.

[15] Janeway, H. T., and Ewing, E.: Ann. of Surgery, Feb., 1914, lix, p. 159.

was administered in quantities sufficient to cause marked respiratory embarassment, demonstrate the readiness with which an asphyxial hyperglycaemia may arise and the efficacy of our method in preventing it.

Etherization with tracheal cannula

The animal was kept under ether for three hours, during which time 8 mgm. of strychnine sulphate in 2 mgm. doses was given intravenously. By lightening up on the ether, and stimulating the animal with a series of taps on the nose, he was kept in distinct tremors most of the time. The reducing power of the blood increased from 0.200 per cent to 0.377 per cent.

Etherization with insufflation tube

Animal was under ether two hours, 4 mgm. of strychnine sulphate was given. Tremors were maintained throughout the period. Reducing power of the blood showed no change, being 0.101 per cent at the beginning and 0.098 per cent at the close.

Operative procedure

The buccal method of exposing the gland was adopted, because it demands the minimum of operative interference, and gives a satisfactory field for stimulation. The use of a dental drill and wax makes a complete exposure of the gland possible in a short time, even in the face of persistent haemorrhage from the bone. The electrodes were plunged through the dura into the gland and stimulation with a tetanizing current applied for twenty to thirty minutes. If the dura is not removed, the chances of recovering the animal for subsequent stimulations are good although no aseptic precautions are taken.

We have studied the stimulation of the gland also by burying iron filings in it, and then exposing them to an electromagnet. Such a method enables us to dispense with an anaesthetic. Our results will form the substance of a later report.

Estimation of the reducing power of the blood

The blood (6 cc. samples) was drawn from the jugular vein into a weighed beaker containing 10 per cent anhydrous sodium sulphate in 1.5 per cent acetic acid, which was immediately reweighed. The beaker was then immersed in a calcium chloride bath at 117° for six minutes, contents filtered, and washed with a definite quantity of the

acid sulphate solution. The reducing power of the filtrate was then estimated by the Bertrand method, the titration being conducted with a permanganate solution previously standardized against a known dextrose solution in the same salt concentration. One cc. of the permanganate was equal to 1 mgm. of dextrose. Occasionally when difficulty was experienced in drawing the samples because of clotting, or when for any reason the washing was not done in the same empirical fashion, the duplicates did not check as closely as one desired. However in no case were there variations sufficient to invalidate the conclusions. We feel that the withdrawal of a sample of sufficient size to give duplicate estimations on aliquot parts would eliminate these variations, but it would also involve the removal of 25 per cent more blood, a factor not to be overlooked.

C. RESULTS

1. The effect of drilling to expose the gland

It was early evident that the mere act of drilling over the gland in the course of its exposure caused a rise in the reducing power of the blood. With the idea of studying this factor and with the hope of stimulating the gland in the absence of the preliminary rise in the reducing power of the blood, it was decided to remove the bone from a number of animals, allow them to recover, and stimulate the gland at some subsequent date. In the first four the whole roof of the mouth from the optic chiasma to well behind the gland was removed. In five others three holes (one over the gland, and the others in front and behind it), separated by bony bridges were drilled in position. A summary of the results follows in Table I.

It thus appears that where the manipulative procedure was extensive ("Exposure of the whole roof of the mouth"), there was a rise in the reducing power of the blood; but where it was not so extensive ("Three holes"), the rise did not always follow (5, 6, 7).

2. The effect of stimulation of the gland

A glance at Table II shows the unmistakable rise in the reducing power of the blood following stimulation of the gland, and that this rise is not dependent upon the drilling is evidenced by dogs 3 and 15.

In only one case (dog 9) was there no rise in the reducing power of the blood. This animal was suffering from the distemper, and had

eaten little or nothing during the previous week. In dog 6 the rise is not so marked as in the other cases. This result may be attributed to the annoying oozing of fluids into the field so that it was impossible to get a clean-cut stimulation of the gland.

3. The effects of stimulation anterior to the gland

An analysis of Table III, which summarizes the results of stimulation anterior to the gland shows that in 3 of the dogs (2, 7, and 17)

TABLE I

Effect of drilling on the reducing power of the blood

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PER CENT OF GLUCOSE		
	Before calling	After drilling	20-30 min. rest
Exposure of the whole roof of the mouth			
1	0.086	0.121	
	0.088	0.115	
2	0.113	0.109	0.160
	0.117	0.108	0.170
3	0.161	0.185	
	0.157	0.187	
4	0.168	0.190	0.192
	0.155		
	Three holes		
5	0.042	0.050	0.050
	0.052	0.049	0.041
6	0.110	0.143	0.134
	0.135	0.146	0.143
7	0.062	0.067	0.076
	0.069	0.069	0.073
8	0.190	0.210	0.233
	0.199	0.204	0.229
9	0.084	0.150	0.160
	0.090	0.160	0.164

there was no rise in the reducing power of the blood, in the others (16, 18, 19) there was a rise of a much lower grade than that found in the cases where the gland was stimulated. Autopsies of the animals with the electrodes in position showed their locations from 0.5 to 3 mm. anterior to the hypophysis.

TABLE II

Effect of stimulation of hypophysis with tetanizing current

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PER CENT OF GLUCOSE			
	Before drilling	After drilling	Stimulation 20–30 min.	Rest of 20–30 min.
2	0.175	0.211	0.237	0.247
	0.183	0.206	0.238	0.245
3	0.072	*	0.196	0.198
	0.082	*	0.196	0.197
6	0.115	0.139	0.171	0.166
	0.119	0.130	0.165	0.169
9	0.053	*	0.055	0.042
	0.044	*	0.048	0.039
10	0.216	0.297	0.488	0.313
11	0.160	0.210	0.180	0.210
12	0.096	0.132	0.160	0.160
13	0.155	0.193	0.226	0.217
	0.165	0.203	0.230	0.217
14	0.176	0.202	0.206	0.214
	0.172	0.198		0.213
15	0.133	*	0.216	0.255
	0.140	*	0.206	0.251

*Previously drilled out.

TABLE III

Effect of stimulation anterior to the hypophysis

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PER CENT OF GLUCOSE			
	Before drilling	After drilling	Stimulation 20–30 min.	Rest of 20–30 min.
2	0.189	*	0.171	0.176
	0.200		0.165	0.182
7	0.053	*	0.065	0.066
	0.061		0.067	0.067
16	0.139	0.143	0.177	0.161
17	0.062	0.062	0.085	0.080
	0.054	0.060	0.098	0.077
18	0.046	0.037	0.078	
	0.035	0.030	0.073	
19	0.111	0.149	0.169	0.170
	0.094	0.148		0.182

*Previously drilled out.

4. The effect of stimulation posterior to the gland

In only one case of Table IV (dog 20) was there a significant rise in the reducing power of the blood on stimulation applied at a point posterior to the gland. The technique of experiments 2, 5, 6, and 9 was all that could be desired, for the field had been previously uncovered and the gland was insulated from escaping currents with dental wax. These results and those obtained from the stimulations anterior to the gland show in our estimation that it is possible to stimulate the floor

TABLE IV

Effect of stimulation posterior to the hypophysis

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PER CENT OF GLUCOSE			
	Before drilling	After drilling	Stimulation 20–30 min.	Rest of 20–30 min.
2	0.193	*	0.167	0.203
	0.201		0.167	0.190
5	0.072	*	0.067	0.061
	0.066		0.061	0.060
6	0.110	*	0.125	0.137
	0.102		0.128	0.137
9	0.096	*	0.061	0.070
	0.089		0.067	0.071
20	0.250	0.255	0.280	
	0.246		0.295	
21	0.100	0.107	0.095	
	0.085	0.095	0.103	

*Previously drilled out.

of the brain in the vicinity of the hypophysis without increasing the reducing power of the blood.

Autopsies confirmed the locations of the stimulations as from 2 to 4 mm. posterior to the gland.

5. Stimulation of the gland in animals whose splanchnic nerves have been previously cut

A table (V) is appended summarizing the results of the stimulations of the gland in animals whose splanchnic nerves had been previously sectioned. Attention is called to the fact that with one exception (22) the nerves had been cut from thirty to one hundred and thirty-seven

days before the experiment. These animals with the exception of 26 had been confined within the cages and had not been allowed the run of the paddock, hence they should have had a supply of "available glycogen" (Weed, Cushing, and Jacobsen). Dog 27 had in addition a double vagotomy, below the heart and above the diaphragm, performed five months previous, so we feel certain that his liver was free of all connection with the central nervous system. The only suggestion of a rise is found in cases 22 and 24, but this is not at all comparable to that found in normal hypophyseal stimulation.

TABLE V

Effect of stimulation of the hypophysis in dogs whose splanchnic nerves have been previously sectioned

ANIMAL	REDUCING POWER OF THE BLOOD EXPRESSED IN PER CENT OF GLUCOSE				
	Before drilling	After drilling	Stimulation 20 min.	Rest 20 min.	Days after section of splanchnics
22	0.107	0.125	0.120	0.125	10
			0.130	0.125	
23	0.074	0.059	0.059	0.055	75
	0.075	0.062	0.052	0.055	
24	0.114	0.116	0.131	0.129	60
	0.114	0.109	0.132	0.137	
25	0.115	0.064	0.071		90
	0.105	0.075	0.078		
26	0.088	0.077	0.031	0.053	30
	0.098	0.087	0.039	0.043	
26	0.078	*	0.062	0.058	70
	0.073			0.065	
26	0.102	*	0.049	0.045	137
	0.101		0.041	0.039	
27	0.056	0.056	0.051	0.056	108
	0.051	0.054	0.050	0.064	

*Previously drilled out.

6. Glycosuria in relation to the reducing power of the blood

Having in mind the diuretic effects of gland extracts, we felt that the glycosuria might be partially attributable to the lowering of the threshold of the kidney for sugar, a phloridzin effect, so simultaneous estimations were made on the reducing power of the urine and blood in thirteen cases. Of seven cases so studied, which did not present glycosuria, the maximum values of reducing power of the blood were 0.110,

0.110, 0.133, 148, 160, and 177 per cent. In those cases presenting sugar, the lowest levels were 0.191 and 0.21 per cent. These values agree well with the threshold as determined by Pollak[16] for rabbits under conditions of diuresis. For a diuretic 10 per cent sodium sulphate was used in 5 to 10 cc. doses depending on the size of the animal.

After the establishment of glycosuria, the sugar increased rather rapidly as the result of stimulation of the gland, and was continued into the succeeding rest period. As an illustration a protocol of dog 13 may be cited.

Dog 13

After anaesthesia, blood reducing power, 0.159 per cent; urine, 0.87 per cent.
After drilling, blood reducing power, 0.190 per cent; urine, 3.96 per cent.
After stimulation, blood reducing power, 0.228 per cent; urine, 5.12 per cent.
After rest of 20 minutes, blood reducing power 0.217 per cent; urine 5.78 per cent.

Macleod[17] in studying the behavior of glycosuria resulting from stimulation of the splanchnic nerve observed that, having once established itself, the sugar in the urine increased out of proportion to that in the blood, and that its return to a normal occurs at a later period. These results suggest a toxic action exerted by the sugar on the kidney cells. Or we may be dealing with a question of habit formation as shown by Mostrom and McGuigan[18] for strychnine convulsions in frogs. Once the kidney cells have responded to a given level of sugar in the blood, they appear to continually lower their threshold with increasingly larger quantities of sugar in the urine. Indeed the clinical therapeutic measure of establishing higher degrees of tolerance in diabetic cases points in the same direction. While we have not followed out the return to normal in hypophyseal glycosuria, and while we feel that our data is too meager to be conclusive, we wish to point out the apparent analogy of this glycosuria to that resulting from splanchnic stimulation.

D. DISCUSSION AND CONCLUSIONS

We feel that the evidence presented in the foregoing section establishes that it is possible to stimulate in the vicinity of the hypophysis without causing a rise in the reducing power of the blood. That stimulation

[16] Pollak, L.: Arch. f. Exp. Path. u. Pharm., 1909, lxi, 157.
[17] Macleod, J. J. R.: Loc. cit., p. 46.
[18] Mostrom, H. T., and McGuigan, H.: Journ. Pharm. and Exper. Therap., 1912, iii, 515.

of the gland causes a rise in the reducing power of the blood in dogs may be regarded as settled. We believe further that any stimulation in this region must be effective only in proportion as it throws the gland into activity. Of course our evidence on this point is not logically complete, for we have not examined every point outside of the hypophysis, nor were all of our experiments on locations anterior to the gland negative. However it was not always possible to produce technically perfect experiments on account of oozing fluids, which must have served as conductors of the currents. The really important question is not whether 50 per cent of our experiments were positive or negative, but whether we were able as the result of improved technique to get a sufficient number of undoubted stimulations in the neighborhood of the hypophysis, which did not cause a rise in the reducing power of the blood. Our protocols establish this clearly.

The drilling constitutes a mechanical stimulus to the gland, and the rise in the reducing power of the blood is explained on this basis. The height attained is less than under electrical stimulation, hence it is not so efficient a stimulus. If the drilling is done carefully, quickly, and with no trauma to the hypophysis, the rise may be absent.

Our results with dogs, whose splanchnic nerves have been cut, are diametrically opposed to those of Weed, Cushing, and Jacobsen on cats and rabbits with cord sectioned at the fourth thoracic vertebra. They are of the opinion that the stimulation of the gland is responsible for the liberation of an internal secretion, which produces glycosuria through a hyperglycogenolysis, since all nervous connections to the liver have been severed. A glance at many of their protocols shows that the stimulations followed within two or three days of the section of the cord. In one instance (XXVIII) although the urine of the animal showed a positive Fehling's at 8.30 a.m., yet a negative one at 12.45 p.m. was deemed a sufficient precaution to allow of etherization and stimulation of the gland at 1.30 p.m. The question arises whether the instability of the blood pressure regulating mechanism has been carefully enough considered. In the absence of proof to the contrary two or three days' time appears a rather short interval to allow for recovery of vaso-motor tone. On this point Sherrington[19] has to say,

> When in the dog complete transection of the spinal cord through the eighth cervical segment is practiced, a severe fall in the general arterial pressure ensues, and vasomotor reflexes cannot be elicited. But in the course of *some* days this is

[19] Sherrington, C. S.: Integrative action of the nervous system, 1911, p. 241.

largely recovered from, and after *some* weeks the blood pressure will, with the animal in the horizontal position, often be found practically normal.

A dog with splanchnics cut one to two months previous and in good health seems to us a more reliable experimental animal than a cat or rabbit with a sectioned cord. Hill[20] states that tone is completely restored to the splanchnic area some eight days after section of all of the splanchnic nerves.

While our results on their face appear to speak against the liberation of a hormone causing the hyperglycaemia, yet this does not necessarily follow. Such a hormone might find its site of action on the terminations of the splanchnic nerves or on the neuro-cellular junction, either in the adrenals or in the liver. If this is the solution of the case, then sectioning of the cord as practiced by Weed, Cushing, and Jacobsen ought to have no effect on these terminations; while our division of the splanchnics would in all probability destroy the fibers to the adrenals, but might not interfere with the activity of those to the liver. For in the case of the adrenal the medulla originates from the same blastema as the peripheral sympathetic ganglia, and thus might be looked upon as the homologue of the postganglionic fiber. On this basis our cut would have destroyed the one neuron to the adrenal. The fibers to the liver relay in the great plexus intimately associated with the branches of the aorta, so our section has destroyed only the pre-ganglionic fibers, leaving the postganglionic ones intact. It has been further suggested to us[21] that the site of action of the hormone might be some central glycogenic center, which required an intact nervous pathway to the viscera for its discharges. We are justified however in concluding that our experiments speak against a hormone, which affects the liver and muscle cells directly causing an increased glycogenolysis. In contrast to the hormone regulation the possibility of a more or less direct nervous route from the hypophysis to the glycogen stores offers itself as an obvious mechanism which will have to be investigated.

CONCLUSIONS

Our results may be summarized as follows.

(1) If precautions are taken to avoid asphyxia, an animal may be anaesthetized with ether without causing a marked rise in the reducing power of the blood. Such a level once established not only does not tend

[20] Hill, L.: Shaeffer's textbook of physiol., 1900, ii, 138.

[21] Dr. A. J. Carlson, University of Chicago.

to increase but generally falls under one to three hours of insufflation anaesthesia.

(2) Electrical stimulation of the hypophysis in dogs under insufflation anaesthesia gives rise to an increase in the reducing substances in the blood. Drilling over the sella stimulates the gland mechanically, but not so efficiently as the induced shocks.

(3) If the stimulation is applied anteriorly or posteriorly to the gland, with precautions to prevent an escape of the current to the hypophysis, no rise in the reducing substances results.

(4) This rise on stimulating the gland does not occur in dogs whose splanchnic nerves have been previously sectioned, a fact which argues against the liberation of a hormone, which increases directly the cellular glycogenolysis.

(5) With active diuresis the threshold of glycosuria lies between 0.190 per cent and 0.21 per cent reducing power of the blood figured to dextrose. Once established the sugar in the urine increases in concentration out of proportion to the reducing power of the blood.

CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXIX. The Gastric Hunger Contractions of the Normal and Decerebrate Guinea-Pig

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INTRODUCTION

Carlson (1) has shown that in the dog and man gastric hunger movements may be modified by psychic stimuli, though only in the direction of inhibition, and that in the dog they continue even when the stomach has been isolated from the central nervous system by section of the vagi and splanchnic nerves. After such section inhibition of tonus and contractions may still be obtained by stimulation of the gastric mucosa, but it is diminished both in intensity and duration. Cutting of the vagi leaves the stomach in a permanently hypotonic condition (2). Without doubt the central nervous system acts merely as one of the regulators of this reflex mechanism but for how much of the regulation the cerebrum is responsible and what part is played by the midbrain and medulla oblongata, can be determined only by observing a decerebrate animal.

Hoping to throw some light on the question, a study of the gastric contractions in normal guinea-pigs was undertaken and subsequently a study of such contractions after removal of the cerebrums. It is well known that this animal survives decerebration for several hours (3) and special reference should be made to the observations of Brown after unilateral (4) and complete (5) removal of the cortex. Sherrington (6) includes the guinea-pig among the animals in which the symptoms of decerebrate rigidity occur with little variation.

PRESENT INVESTIGATION

For observations on the stomach contractions gastric fistulas were made in our animals and the recording method described by Carlson (7) was employed. Sixteen animals weighing from 450 to 790 grams were studied over periods varying from 13 to 66 days. The guinea-pig is so foreshortened that the operation was beset with some difficulties—the fundic portion of the stomach is pushed up under the diaphragm in such a manner that it must be pulled downward and stitched to the abdominal wall to make a fistula, or an opening must be in the pyloric region. Both methods proved satisfactory and the possible objection to the lower opening that the balloon did not lie in the fundus was obviated by the use of a small balloon or of a finger cot from 3 to 5 cc.

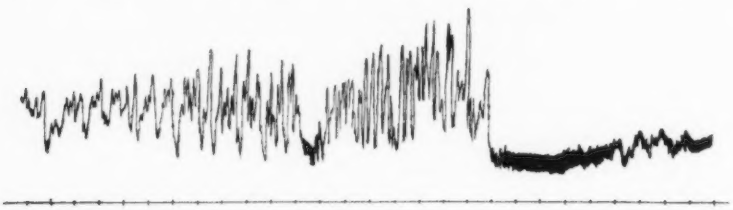

Fig. 1. Tracing showing the termination of a period of gastric hunger contractions of a guinea-pig, five hours after feeding. Note the gradually increasing intensity of the contractions and the incomplete tetanus which ends the period. Three hours later, when the balloon was removed, the stomach contained food. Time: 27 minutes.

This and the following figures were retraced from the original tracings by the author.

in capacity, pushed well up into the stomach. Several animals killed with the balloon in place, left no doubt of the ease with which it was properly inserted. Very small fistulas were made so that when the wounds healed they often measured less than a centimeter across. Since the animals began eating within twelve hours, the food in the distended stomach prevented the openings from closing. A dressing was unnecessary after the fourth or fifth day but vaseline was applied daily during the observation period. In recording a water manometer was used, the pressure varying between 3 and 4 cms.

The guinea-pig, like other herbivorous animals, feeds at frequent intervals—probably every hour—and under normal conditions the stomach is never found empty. Even within two hours after exclusion

from food it begins eating its own excreta, a fact already reported (8), and after twelve hours will eat paper, pasteboard or anything of that nature within reach. The easiest and most effective method found for excluding it from its own feces was to place all but the head in a bag, sufficiently small to prevent much freedom of movement, and then to draw the bag closely about the neck.

Rogers (9) has reported the appearance of gastric hunger contractions in the rabbit twelve hours after its exclusion from food and excreta. In the guinea-pig a similar type of contraction was recorded in five hours (fig. 1). Frequently continuous records were made from the time the food was removed until the onset of such vigorous movements. The mild peristaltic waves become more and more intense until contractions such as might be classified as type I (2) appear—periods of feeble tonus lasting two or three minutes with four or five superimposed contractions. This type may continue for four hours but, as in figure 1, they gradually merge into the more vigorous type II and possibly III. The contractions follow one another in rapid succession—one in eighteen seconds (average)—such a period terminating in complete quiescence of the stomach. At times a period of violent coughing precedes the inhibition. Contractions of types I and II have been recorded continuously for six hours with but two periods of rest lasting eight and six minutes respectively. In figure 1 the short period of inhibition was followed by contractions of gradually increasing intensity which continued for three hours when the record was discontinued on account of the animal's restlessness.

The subject's voluntary movements—turning about and licking itself—may cause the termination of a hunger period. Sudden noises or talking close by usually have an inhibiting effect though records started amid the noise of a general laboratory were sometimes as satisfactory as those secured with quiet surroundings. Substances which cause inhibition when placed in the stomach of man or the dog or when they stimulate the end-organs of taste, are without effect on the guinea-pig. Water was introduced, 0.5 per cent hydrochloric acid, sugar solution, and quinine by a pipette into the mouth, some was always swallowed though probably scarcely half a cubic centimeter. All these substances, like the acid (fig. 2, *a*) were negative in their effect, whether the contractions were mild or intense. It was difficult to introduce substances directly into the stomach without attracting the animal's attention, for the presence of an extra tube passing through the fistula usually proved irritating and prevented the onset of hunger

movements. In a few instances, however, we succeeded in introducing about 1 cc. of the various substances but with negative results. Smelling and tasting food—when the animal remained quiet—produced no inhibition but we were surprised to find that even the chewing and swallowing of the most appetizing substances such as clover or lettuce also gave negative results (fig. 2, *b*). To be sure a hungry animal frequently became so restless as soon as food was given that further observations were impossible but such a record as the one shown, taken during the eleventh hour of fasting, has been repeatedly obtained. Vigorous contractions like those at either end of this tracing had gone on for nearly four hours without inhibition. Between the arrows the guinea-pig quietly but eagerly ate lettuce. The movements continued for ten minutes then it was allowed to eat for five minutes, the same type of contraction was recorded for fifteen minutes, following which the subject became very restless and the observations were discontinued. In other instances more food was given and the onset was watched of normal digestive peristalsis, occurring after about half an hour. These observations on the difficulty of inhibiting the hunger movements are in agreement with those reported for the rabbit (9), are indeed even more pronounced. An animal fasted for such periods as twenty-four and forty-eight hours was exceedingly restless and long continued records were not possible, but the periods of increased tonus or tetany are more frequent than during the earlier hours.

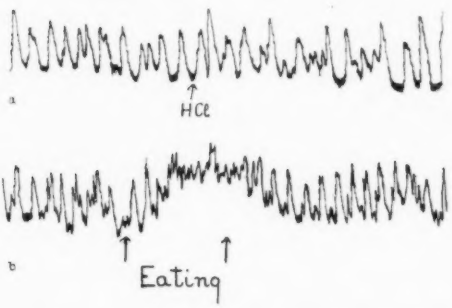

Fig. 2. Tracing showing hunger contractions of guinea-pigs—*a*. Failure of 0.5 per cent HCl, given by mouth to cause inhibition. Time: 11.5 minutes. *b*. Failure of chewing and swallowing food to cause inhibition. Time: 16.5 minutes. In both instances the stomach contained food.

An interesting peculiarity and one of some significance, is the periods of inhibition often noticed in the midst of normal digestion peristalsis (fig. 3). They are of about the same duration as those terminating a hunger period—eight to ten minutes—and come on when there are no outside disturbing factors. Their occurrence emphasizes the close

relation existing between the movements of the stomach during digestion and those during hunger. Rogers' (9) observation for the rabbit that "gastric hunger contractions are intensified peristalsis" may undoubtedly be made for the guinea-pig as well.

That discomfort is experienced when food is withheld for even four or five hours is evidenced by restlessness, the eating of the animal's own excreta, chewing movements and sometimes crying when the contractions are unusually vigorous. But why such hunger should be experienced while the stomach still contains abundance of food (it not only adheres to the balloon when removed but comes from the fistula) is difficult of explanation. The temperature of the normal guinea-pig taken by fistula is 103.2° F., perhaps the active metabolism of the animal, due to its small size and almost constant activity, explains its constant need of food.

EFFECT OF DECEREBRATION ON THE HUNGER CONTRACTIONS

Since psychic inhibition plays such an unimportant rôle in the control of the hunger mechanism of the guinea-pig, we did not expect to find the stomach movements modified by removal of the cerebrum—when time had been allowed for recovery from the shock of the operation. Ten animals were decerebrated, none lived less than eighteen hours, some nearly thirty-six and one was killed at the end of seventy-five hours. The lesions were made by a thermocautery so that excessive hemorrhage might be prevented. Later the lesions made were located by sectioning the brains and mounting every tenth section, or by examination of the gross sections. The cortical lesion, in nearly every instance, was so extensive as to leave no doubt that its functional significance was destroyed. The corpora striata were penetrated, in some cases much more extensively than in others, and in a few the lesion included a portion of the optic thalami.

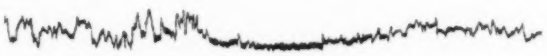

Fig. 3. Tracing showing the inhibiting of normal digestion peristalsis in the undisturbed guinea-pig. Compare this with the similar period in figure 1. Time: 13 minutes.

A rather detailed description of the symptoms of one animal may be taken as typical.

The guinea-pig was taken from its food and quickly decerebrated.

By the time the wound was closed it was able to walk about and exhibited the usual symptoms—repeated shaking of the head, restlessness, struggling when restrained and very rapid rate for heart and respiration (these could not be counted). A little later other observations were made—it would start violently when the skin was pinched or the leg pulled, would fall from the table when allowed to wander freely about, or would beat its head against the sides when placed in the cage. No notice was taken of objects passed before its eyes unless the lashes were touched, if the hands were clapped close by, the ears were moved as in the normal animals. It struggled and cried when the balloon was inserted—normal animals offer very little resistance. During the first day, periods of quiet were broken by extreme restlessness—turning about, licking itself, biting at the edge of the box or at the fingers of the investigator. After twenty-four hours the animal was less active and would sit for an hour at a time with neck drawn in and hair roughed up, resembling the attitude of a decerebrate pigeon. On the third day it was very weak and lay on its side much of the time while records were being taken, its limbs were rigidly extended and trembling. During the night succeeding it was observed at frequent intervals, being more active than during the day, the animal frequently climbed from its box and would be found lying on its back or side struggling aimlessly, or suspended on the edge unable to get in or out. On the fourth day it lay on its side much of the time and was killed at the end of seventy-five hours. During the period of observation water was frequently given by the fistula, a little could be swallowed if placed far back on the tongue by a pipette. An examination of the brain showed complete destruction of the cortex on the dorsal and lateral surfaces, the cortex of the hippocampal lobes and the olfactory lobes was not injured. The lesion penetrated the corpora striata and the thalami.

Some of the animals were much more active than the one described and were able to jump from a box a foot deep. Most of them exhibited spasmodically the symptoms of decerebrate rigidity—limbs extended and head drawn backward. In all cases records were obtained in spite of the excessive activity. In six there were periods of quiet continuing for half an hour or more. Gastric movements were recorded, in the guinea-pig described above, six hours after the operation and figure 4 (*a*), the close of the seventh hour, shows the type of contraction. The subject was remarkably quiet and the inhibition which terminated the hunger period had lasted for four and a half minutes, then mild peristaltic contractions set in which merged into those of type I. These

continued for half an hour when the animal became so active that the inflated balloon was pulled from the stomach. Abundance of food adhered to it and came from the fistula. The marked degree of tonus shown in these contractions characterized those of the decerebrate guinea-pig. The tonus periods (fig. 4, *a*) lasted from a minute to one and a half and a superimposed contraction appeared every ten seconds—twice as frequently as in the normal. In this animal the tonus was less pronounced after the first day (fig. 4, *b*), although the contractions occurred at the same rapid rate, but others showed it in unusual degree for twenty-four and thirty hours. The tonus continued but the gastric movements gradually became less vigorous until on the fourth day when only respiratory movements and very feeble contractions were recorded. Water, etc., given by the mouth caused no inhibition, no attempt was made to introduce it directly into the stomach while recording.

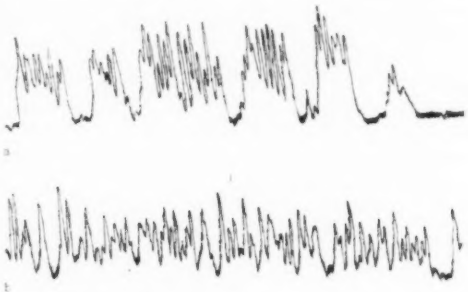

Fig. 4. Tracings showing contractions obtained from a decerebrate guinea-pig: *a*. Termination of a hunger period 7 hours after the operation, 7.5 hours after feeding. Note the marked periods of tonus. Stomach contained food. Time: 11 minutes. *b*. Hunger contraction from same animal, 25 hours after operation. Stomach empty.

The striking variations, then, of the decerebrate from the normal animal are—an increased tonus of the musculature of the stomach and an increase in the rate of contraction. For the normal these average two to three per minute as opposed to six in the operated animal. Periods of inhibition were usually briefer than in the normal but as to the frequency of their occurrence an accurate statement is impossible, for hunger contractions were so often terminated by the animal's activity. The restlessness of the decerebrate guinea-pig as of Goltz's dog, may be attributed to the absence of inhibitory impulses from the cerebral cortex. In the normal animal such impulses must be continually influencing the gastric movements—decreasing their rate and lowering the muscular tone. Since section of the vagi in the dog (2) leaves the stomach in a hypotonic state and removal of the cortex, in the guinea-pig at least, is followed by

a condition of hypertonus we may infer that impulses from centers in the mid-brain and medulla and not those from the cerebrum exercise the controlling influence.

SUMMARY AND CONCLUSIONS

1. Hunger contractions, comparable to those observed in other animals, appear in the guinea-pig from four to five hours after feeding, while the stomach is still well filled.

2. Water, 0.5 per cent hydrochloric acid and other substances which usually cause inhibition of such contractions give negative results, whether swallowed or placed directly in the stomach. After eating, the vigorous movements continue for about half an hour and then merge into those of the mild peristaltic type.

3. The normal peristaltic movements of digestion may be interrupted by intervals of quiescence such as terminate a hunger period. This fact adds additional weight to the suggestion of Carlson that hunger contractions are simply more vigorous peristaltic movements.

4. After decerebration, contractions of a similar character are recorded but showing a marked increase in rate, the stomach being in a hypertonic condition. It is held that the absence of inhibitory impulses from the cerebral cortex accounts for these striking variations, the positive influence of the brain on stomach motility originating below the cerebrum.

The problem of this study was suggested by Professor Carlson and his kindly interest and criticisms of the work are gratefully acknowledged.

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